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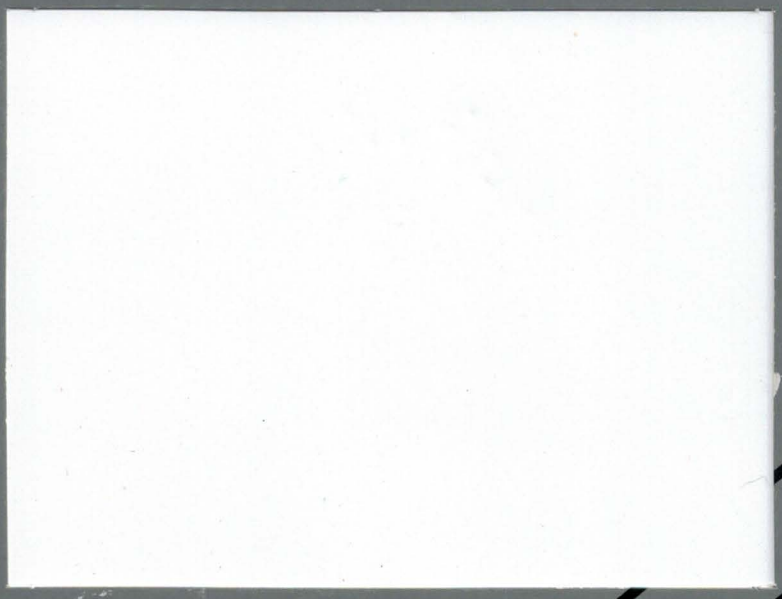

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REPORT BRIEF

Railroad Industry Research Needs for *In Situ* Bioremediation

R-858

May 1994

One of the most pervasive environmental remediation problems facing the railroad industry is contamination of soil with diesel fuel. *In situ* bioremediation provides specific benefits as a remediation approach to the railroad industry, including limitation of future liability, ability to treat soils and groundwater beneath active railroad facilities, and cost effectiveness.

The Research and Test Department's Environmental and Hazardous Materials Research Division undertook a research project to investigate and evaluate the state of the art of *in situ* bioremediation as an option for subsurface remediation of petroleum hydrocarbon contaminated soils. Railroad Industry Research Needs for *In Situ* Bioremediation is designed to present specific technical and regulatory issues that need to be addressed to improve the efficient application of this remediation technology for railroad industry sites.

In order to improve the reliability, performance, and economics of *in situ* bioremediation, there is a need for research in the areas of microbiological, hydrogeological and geochemical aspects, process monitoring and documentation, and engineering options. Microbial issues include understanding the bioavailability of contaminants, optimizing nutrient requirements, and understanding the mechanisms involved in bioaugmentation. The applicability of *in situ* bioremediation could be expanded through improved understanding of the relevant hydrogeological and geochemical limitation in order to avoid iron precipitation and enhance subsurface permeability. Being able to validate the occurrence of *in situ* bioremediation by process monitoring and documentation is critical for its optimization and acceptance. Finally, various engineering options proposed to enhance bioremediation systems require further research. These include aquifer aeration, use of surfactants, and temperature control.

A thorough review of both published and unpublished literature has revealed four major areas in which fundamental research is required to further advance the potential for *in situ* bioremediation:

- Qualification of biodegradation kinetics - research is needed to separate biotic and abiotic processes and derive kinetic rate constants for various reaction conditions.
- Dissolution and desorption kinetics - research is needed to understand mass transfer limitations and dissolution and desorption kinetics.
- Biologically induced clogging of the subsurface - research is needed to determine primary clogging mechanisms and examine methods for prevention, minimization and management.
- The transport of colloids - research is needed to increase the understanding of subsurface transport of microbial populations and substrates, in the form of colloids.

Based on a review of the current regulatory climate, several research opportunities exist in order to improve regulatory understanding and acceptance of the capability and control of *in situ* bioremediation. Movement towards risk-based environmentally acceptable cleanup levels requires a greater understanding of the mobility and toxicity of the contaminants, the loss of contaminant mass due to biodegradation, and the site-specific contaminant exposure mechanisms. Also, a generally accepted protocol is needed that will verify the occurrence and effectiveness of *in situ* bioremediation. Third, in order to evaluate the appropriateness of the technology for a site, a complete and thorough site characterization is necessary and development of a standard protocol is needed. Fourth, alternative techniques for delivery of additives to the subsurface need to be developed. Finally, development of a data base of case studies, and expert systems to provide information on past performance and engineering of new systems, would provide current and accurate performance data.

Orders for AAR Report "Railroad Industry Research Needs for In Situ Bioremediation" should be sent to: Association of American Railroads, Publication Order Processing, 50 F Street, NW - 5th Floor COG, Washington, DC 20001. The AAR Report number is R-858. The price is $10.00 for member railroads and $100.00 for nonmembers. Illinois residents add 6.25% (Chicago--8.75%) sales tax. Price includes domestic shipping and handling charges. There will be shipping charges for locations outside the United States. Checks should be made payable to the Association of American Railroads. Visa and Mastercard are accepted for payment. A complete listing of reports is available upon request.

Association of American Railroads

Research and Test Department

RAILROAD INDUSTRY RESEARCH NEEDS FOR *IN SITU* BIOREMEDIATION

Report No. R-858

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April 1994

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EXECUTIVE SUMMARY

The Railroad Industry Guide to Research Needs for *In Situ* Bioremediation (the Research Guide) is the second of two documents that address the use and development of *in situ* bioremediation for treating diesel contaminated soils at railroad properties and facilities. The Research Guide identifies and evaluates research needs related to *in situ* bioremediation. This report is designed to present specific technical and regulatory issues that will assist in prioritization of the railroad industry's *in situ* bioremediation research efforts.

Chapter 1 states the purpose of the Research Guide, and presents an overview of possible applications of this report in providing a framework and plan for research related to *in situ* bioremediation. A definition of *in situ* bioremediation is presented in Chapter 1 to familiarize the reader with the focus of the research efforts.

Chapter 2 summarizes a series of topics which are potential areas for future research related to the technology, and introduces research opportunities. Topics considered include microbiological issues, hydrogeological and geochemical issues, process monitoring and documentation, and engineering options.

Research needs as defined from a review of the technical literature are discussed in Chapter 3. A brief scope of the literature review and the technical approach for its execution are described. The results and trends identified in the literature review are summarized, the their implications on the use of *in situ* bioremediation by the railroad industry is presented.

In contrast to Chapter 3, Chapter 4 identifies research needs that are driven by regulatory concerns and needs. This chapter briefly summarizes a series of topics which are potential areas for future research, and which may offer opportunities to improve regulatory understanding and acceptance of the capability and control of *in situ* bioremediation. Topics considered include: 1. environmentally acceptable treatment end points; 2. system performance measurement; 3. site and contaminant characterization; 4. delivery to the subsurface environment of oxygen and other treatment amendments; 5. case studies; and 7. expert systems.

The final chapter, Chapter 5, provides a summary of the research needs defined in the Research Guide. Priorities for future research efforts are identified.

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The effort that goes into creating any report is not exerted solely by the names appearing on the cover. Therefore, there are a number of people and organizations who need to be recognized for their contributions.

Our appreciation is extended to the environmental representatives of the Class I railroads in North America who participated in our survey and whose opinions are reflected in this report. Dr. Ray Loehr provided peer review of the report and Darcy Magee of Remediation Technologies, Inc. ably acted as project manager, binding together the widespread actions of many people and directing the report to completion. Finally, Dee Ann Campbell of Renaissance Editing provided valuable technical editing and production services.

1.0 INTRODUCTION

The Association of American Railroads (AAR) has developed a set of reports which outline the state of the art of *in situ* bioremediation of saturated and unsaturated soils. Specifically, the reports address the current and developing technologies as they pertain to diesel-contaminated soils in typical railroad situations. The distinction between the two reports is in their emphasis: A Guide for Railroad Industry Use of *In Situ* Bioremediation (the User's Guide) defines when, where, and how to implement bioremediation technologies; Railroad Industry Guide to Research Needs for *In Situ* Bioremediation (the Research Guide) presents specific technical and regulatory-driven issues designed to assist in prioritizing the railroad industry's *in situ* bioremediation research efforts.

1.1 WHAT IS *IN SITU* BIOREMEDIATION

For the purposes of this report, bioremediation is defined as the engineered enhancement of the conversion of organic contaminants into biomass and harmless byproducts of microbial metabolism, such as carbon dioxide, methane, inorganic salts, and water. *In situ* means "in place," which, for the purposes of this report, is interpreted to include the unsaturated (vadose) and saturated zones of the subsurface. Thus, *in situ* bioremediation is an engineered approach to subsurface remediation in which concentrations of organic contaminants in soils and groundwater are reduced within the subsurface environment through the activity of naturally-occurring microorganisms. Although some *in situ* bioremediation techniques may involve the extraction of contaminated groundwater or soil vapor, which may then require treatment through surface treatment systems, excavation of contaminated soils is avoided and the majority of contaminant reduction occurs "in place."

1.2 REASON FOR AND PURPOSE OF REPORTS

1.2.1 Need for Remediation

Diesel fuel has been the principal fuel for railroad locomotives since the early 1950s. Diesel locomotive fueling operations, diesel fuel storage, and transportation, have resulted in the release of diesel fuel to soils and groundwater at railroad facilities across the country. It is estimated that over 150 major active fueling and fuel storage facilities are associated with the railroad industry in North America. Many of these facilities are 20 years old or older and some have experienced leaks or spills to soil or groundwater during their operating life. Former or inactive fueling facilities account for another 150 to 200 sites nationally. In

addition, derailments and other accidents have resulted in the release of diesel fuel to the soil and groundwater.

With the heightened environmental awareness within the railroad industry, these releases have become the subject of concern. The railroad industry is currently in the process of replacing many storage tanks and fueling systems and, as part of that effort, is often required to manage contaminated soils and groundwater. The remediation of diesel-contaminated soils associated with derailments and spills is also a routine concern of railroad environmental personnel.

Soil and groundwater contamination at railroad facilities and on railroad property is often located under, or adjacent to, tracks, maintenance facilities, and other structures. Remediation efforts in these areas must be conducted so as not to disturb those facilities or interrupt traffic over the track or otherwise interfere with railroad operations which provide a valuable service to American industries. Extensive excavation to remove contaminated soils, and techniques to cap or otherwise isolate contaminated soils, can cause substantial disruption to vital railroad operations. Because of these factors, *in situ* remediation alternatives are applicable to many railroad-related contamination sites. The additional time required to effectively implement *in situ* remediation is an acceptable compromise when significant disruption to track facilities can be eliminated.

1.2.2 Purpose and Design

Each of these reports has been designed to serve a specific purpose. The User's Guide will serve as a user's handbook to the evaluation and implementation of *in situ* bioremediation for the treatment of petroleum-contaminated soils and groundwater. The Research Guide will be used to identify and focus future AAR *in situ* bioremediation research efforts and will serve to coordinate those efforts with the efforts of other researchers.

The User's Guide

The User's Guide is designed to act as a guide to evaluating and implementing *in situ* bioremediation technologies at railroad facilities. It presents technical and regulatory information that a practitioner can use in evaluating *in situ* bioremediation as an alternative for a specific site or facility. Chapter 2 of the User's Guide presents the basic principles of *in situ* bioremediation technologies. Chapter 3 is a discussion of the regulatory factors impacting the implementation of bioremediation technologies and can be used as a resource

for identifying and solving regulatory issues when evaluating the feasibility of *in situ* bioremediation for both saturated and unsaturated soils. Chapter 4 provides a methodology for evaluating the technology's applicability at specific sites. Chapter 5 presents a summary of literature related to *in situ* bioremediation technologies. The literature summary is provided so that the practitioner may research specific issues which impact the implementation of a particular bioremediation technology. Chapter 6 is a conclusion section and is followed by the references used to prepare the report.

The Research Guide

In situ bioremediation is a rapidly developing technology and numerous technical problems and regulatory issues exist which prevent its wider application within the railroad industry. The railroad industry and AAR have made a commitment to apply resources to solving some of these problems and resolving some of these issues.

This report, the Research Guide, is designed to present specific technical and regulatory-driven problems that are not currently understood, and that significantly impact *in situ* bioremediation at railroad diesel-fuel-contaminated sites. The Research Guide will assist AAR's staff and the Environmental Affairs Committee in evaluating and setting priorities related to the railroad industry's *in situ* bioremediation research efforts. In addition, this report will be used by the AAR staff in evaluating possible participation in joint research efforts with other industrial and public research groups. In short, this report will provide a framework and plan for AAR's research related to *in situ* bioremediation.

Chapter 1 provides an overall introduction to the Research Guide. Chapter 2 outlines the current technology and provides topics for further research. Chapter 3 surveys the available literature covering advances in *in situ* bioremediation technologies. Chapter 4 provides the regulatory issues requiring further research and Chapter 5 summarizes the report.

1.3 CURRENT REMEDIAL APPROACHES

Petroleum-contaminated soils generated by railroad operations are currently managed predominately by excavation. Once contaminated soil has been excavated, it is managed on-site or off-site by either treatment or disposal. The treatment techniques currently used include biological treatment (normally land treatment), thermal treatment, and physical treatment (fixation). Disposal alternatives are limited to land disposal.

Ex situ biological treatment in land treatment facilities has been extensively used for the treatment of petroleum-contaminated soils. Onsite *ex situ* applications have been implemented by railroads at spill sites and fueling facilities nationwide. Performance of these systems has been effective and the systems have generally been cost effective. However, as the cost of thermal treatment is reduced, and becomes competitive with land treatment, this technology becomes less attractive. Commercial land treatment facilities also accept petroleum-contaminated soils.

Thermal treatment is another available alternative for excavated, contaminated soils. Commercially available fixed or mobile thermal units are used to drive the relatively volatile petroleum products from the soil. The thermal units used are normally asphalt kilns which have been modified for the specific purpose of treating contaminated soils. In addition, asphalt plants in several states are licensed to accept petroleum-contaminated soils and incorporate them into asphalt products. Asphalt kilns designed to treat contaminated soils are often fitted with exhaust gas treatment systems such as fume incinerators and particulate removing bag houses or electrostatic precipitators. Exhaust gas control requirements depend heavily on individual state air pollution control regulations. These regulations subsequently, and significantly, impact the treatment cost. Thermal treatment costs range from $30 to $200 per ton depending on the volume of material to be processed, the regulatory controls imposed, and the sophistication of the unit.

Physical treatment to immobilize petroleum contamination in soils has had limited success. Portland cement-based additives are used to stabilize contaminated soils. Other oil-adsorbing materials are also used to ensure that petroleum contained in the soil is not able to migrate.

Landfilling is still permitted in many states and can be a very cost-effective alternative for the disposal of contaminated soils. Petroleum-contaminated soils are frequently used as intermediate cover at sanitary and hazardous waste landfills. Limitations on the concentration of petroleum in the intermediate cover prevent the land disposal of soils containing more than a few hundred parts per million of petroleum. Shrinking landfill capacity prohibits this option in many parts of the country, and the potential future liability associated with hazardous waste landfills also makes the use of this alternative unattractive to many railroads. The disposal costs for landfilling vary from approximately $20 to over $250 per ton, depending on the regulatory status of the facility (Resource Conservation Recovery Act hazardous or solid waste landfills) and market factors.

1.4 WHY *IN SITU* BIOREMEDIATION

Recent advances in the application of biological treatment techniques to petroleum-contaminated soils and groundwater have resulted in a greater understanding of the biological, chemical, and physical processes involved. As a result of lab-scale research and field-scale implementation of biological treatment systems, practitioners have come to understand the potential for biological treatment *in situ*. There are several limitations to the technology, related to site conditions and contaminant characteristics, but within the confines of these constraints, *in situ* bioremediation has proven to be a cost-effective alternative to treatment techniques which require removal of contaminated soils and subsequent isolation techniques.

The primary advantages of *in situ* bioremediation are the ability to remediate soils without excavation and lower costs of biological treatment in general. The disadvantages are the relatively long time-frame required to achieve treatment goals and the inability of the technology to reduce contamination to non-detectable levels.

In situ bioremediation may be an effective alternative at a number of diesel-contaminated railroad sites, because diesel is readily biodegradable and site disruption is minimized. In addition, excavation and development pressures that require fast solutions are not experienced at many railroad properties.

2.0 TECHNOLOGY ISSUES REQUIRING FURTHER RESEARCH

In situ bioremediation is a rapidly maturing technology which draws upon scientific and engineering principles from many technical fields. As a result, researchers and practitioners involved with *in situ* bioremediation continue to find new insights, challenge existing preconceptions and assumed limitations, and pose new questions about the theory and application of bioremediation processes. This section briefly summarizes a series of topics which are potential areas for future research, and which may offer opportunities to improve the reliability, performance, and economics of *in situ* bioremediation approaches. These topics and issues were identified and selected based on issues identified in the literature which indicated the need for future research, and a subsequent analysis by the authors of this report of specific technical and regulatory uncertainties. These topics have been grouped into four general categories associated with the bioremediation process: Microbiological Issues; Hydrogeological and Geochemical Issues; Process Monitoring and Documentation; and Engineering Options. In several instances, this classification is somewhat arbitrary due to the inherent interdisciplinary nature of the field of *in situ* bioremediation.

2.1 MICROBIAL ISSUES

2.1.1 Biomass Production and Nutrient Requirements

Bioremediation is predicated on the incorporation of organic contaminants into new cellular material by the native subsurface microorganisms. Therefore, increasing the growth rate and general metabolic activity of the microbial populations to reduce contaminant concentrations are primary objectives of the bioremediation process. However, when microbial growth results in the production of biomass, or the secretion of extracellular polysaccharides (i.e., "slime"), it can clog the pore spaces of the aquifer, reducing the flow of water and compromising the effective transport of oxygen and nutrients. This is especially true in sites having low permeability. Fouling from excessive microbial growth is particularly common in the vicinity of injection wells and trenches where the microbial populations are exposed to high nutrient and oxygen concentrations.

The relationship between organic loading, nutrient and oxygen supply, and cell yield is well understood in biological wastewater treatment engineering, but the dynamics of this relationship in subsurface environments have not been extensively studied. It is known that under certain nutritional conditions, microorganisms will assimilate organic materials at high

rates without a corresponding increase in biomass production. Additional efforts should be directed toward developing an understanding of the ways that nutrient formulations, concentrations, and delivery techniques can be modified to increase or maintain high rates of contaminant mineralization (conversion to carbon dioxide and water), while minimizing increases in biomass in saturated subsurface environments.

The requirements for inorganic nutrients in unsaturated zone bioremediation (bioventing) are also poorly understood. Several investigators have concluded that nutrients are relatively unimportant to the rate and extent of contaminant biodegradation during bioventing. Nevertheless, it seems likely that nutrient limitation may be a factor in successful *in situ* bioremediation, especially in soils having high contaminant concentrations. Specific research to evaluate the circumstances when nutrient supplementation is required for bioventing processes, and effective techniques for nutrient delivery, is clearly needed.

2.1.2 Alternate Electron Acceptors

Recent studies suggest that nitrate, sulfate, acetate, iron, and carbon dioxide enhance the remediation of petroleum hydrocarbons in the subsurface under certain environmental conditions. Much of the data on the role of these alternate electron acceptors in the biodegradation of organic chemicals have been derived either from laboratory studies that are difficult to extrapolate to the actual subsurface environment, or have been based on observations made during field pilot demonstration projects with inadequate controls to allow for scientifically-defensible conclusions. Studies that have systematically focused on the effectiveness and limitations of alternate electron acceptors for petroleum hydrocarbon biodegradation are relatively scarce. The use of alternate electron acceptors offers distinct potential advantages to traditional approaches to aerobic bioremediation in the saturated zone. For example, the use of nitrate as an electron acceptor takes advantage of the reduced, anaerobic conditions that are typically found in the center of a subsurface hydrocarbon plume. Since anaerobic conditions are maintained in this approach, there is no concern of oxidation and subsequent precipitation of dissolved iron. Additionally, nitrate is highly soluble in water, and is relatively nonreactive with soils. Therefore, the transport of nitrate in the subsurface is much easier than transport of oxygen or hydrogen peroxide. Other alternate electron acceptors may offer similar advantages compared to the use of oxygen. For these reasons, concerted research in the application of alternate electron acceptors offers potential advancements to cost-effective applications of *in situ* bioremediation.

2.1.3 Bioavailability of Contaminants

The accessibility of subsurface organic contaminants to microbial uptake and metabolism is exceedingly complicated, but is of critical importance to effective bioremediation. The availability of these contaminants to microorganisms can be dictated by a variety of physical and chemical processes, including:

- the adsorption of contaminants onto soil particles;
- the concentration of the contaminants; and
- the solubility of the contaminants.

Organic contaminants are subject to adsorption onto soil particles through the combined action of various physical and chemical forces. There is very little information that specifically addresses the extent to which biodegradation processes are compromised when compounds are adsorbed within the soil matrix, and existing information is contradictory. For example, some solid-phase bioremediation studies of petroleum hydrocarbons in clay soils have reported relatively poor reduction of hydrocarbons, presumably due to adsorption of the hydrocarbon compounds onto the fine-grained soils. Conversely, biodegradation processes have apparently been used successfully to regenerate activated carbon in certain wastewater treatment applications. It is important, therefore, that impact of sorption processes on the effectiveness of *in situ* bioremediation be evaluated.

It is well-known from traditional, pure-culture microbiology that, for many organic compounds, the rate at which the compound is metabolized is a function of the compound's concentration. A compound that is rapidly biodegraded at one concentration may be toxic or inhibitory to microbial activity at a higher concentration. Additionally, the same compound may not be degraded at all if the compound's concentration is so low that the microorganism cannot obtain sufficient energy. However, the effect of concentrations of complex mixtures of organic compounds, such as petroleum products, and in diverse subsurface microbial populations, has not been adequately studied. Likewise, it is not known whether poorly-soluble organics, such as high molecular weight polynuclear aromatic hydrocarbons (PAHs), may persist in the environment, because the solubility of these compounds is lower than "threshold concentration" for biodegradation.

The relationship between bioavailability, leachability, and health risk (or toxicity) present additional implications for the remediation of petroleum hydrocarbons. If it can be demonstrated that the leachable components of petroleum products are subject to

biodegradation, while any persistent hydrocarbons that are retained in the soil matrix pose insignificant risks to health or environmental quality, it may be possible to re-evaluate remediation goals for petroleum hydrocarbons and expand the applicability of *in situ* bioremediation.

2.1.4 Addition of Alternate Substrates

The Gas Research Institute has reported that the addition of methane and oxygen into aquifers contaminated with petroleum products can facilitate the biodegradation of benzene, toluene, ethylbenzene, and xylenes (BTEX) compounds to extremely low levels. The implication is that microorganisms will use methane as a primary growth substrate and will co-metabolize BTEX compounds at concentrations well below the threshold at which these compounds can be used by the microbial populations as a primary source of carbon for energy and growth. These studies require confirmation in the laboratory and through field pilot demonstrations. If this approach is proven to be effective, it may have application in situations that require especially aggressive measures, such as when contamination threatens drinking water wells.

2.1.5 Addition of Microbial Products

In certain cases, contaminants may persist in the subsurface because the size of the microbial populations is inadequate, or the resident populations lack the metabolic capability of degrading the constituents of concern. Bioaugmentation (the addition of microbial cultures to the environment) is suggested by many vendors as an effective approach to *in situ* bioremediation; however, there has been very little independent research into questions regarding the survival and transport of introduced microorganisms within the subsurface. It is important to clearly identify the chemical and physical characteristics of the subsurface soil matrix that will make bioaugmentation a potentially feasible and effective alternative for *in situ* bioremediation.

Genetic engineering (altering the genetic material of common indigenous microorganisms to confer the ability to metabolize environmental contaminants as growth substrates), offers great potential for expanding the future use of bioremediation to the treatment of highly persistent contaminants (chlorinated pesticides, PCBs, etc.), although the current stringent controls on the environmental release of genetically engineered organisms are likely to restrict the development of this technology in the short-term. Additionally, the same problems of microbial survival and transport that currently impact the applicability of

bioaugmentation must be addressed before the addition of genetically engineered microorganisms can significantly improve the effectiveness of *in situ* bioremediation.

More promising, perhaps, than the introduction of microorganisms for bioremediation, is the introduction of microbial enzymes to catalyze the degradation of contaminants in the subsurface. The use of enzymes poses none of the transport problems that are likely to complicate the introduction of microorganisms, and also avoids the possibility of excessive biomass accumulation. Despite the potential advantages from the use of enzymes for subsurface remediation, very little laboratory or field research has been performed to evaluate the use of this technology.

2.2 HYDROGEOLOGICAL AND GEOCHEMICAL ISSUES

2.2.1 Iron Precipitation

The precipitation of ferric hydroxide resulting from the aeration of groundwater containing soluble ferrous iron is a common problem in groundwater extraction and treatment projects. When iron precipitation occurs within the subsurface as a result the injection of oxygenated groundwater for *in situ* bioremediation, the permeability of the saturated zone can be substantially decreased, especially in fine-grained soils exhibiting low initial permeability. Currently, however, few laboratory or field methods have been evaluated that adequately predict the extent to which iron precipitation will impact aquifer permeability and groundwater flow at a specific site. It may also be possible to avoid iron precipitation through chemical additives and/or flushing with anoxic water. Research into the prediction and avoidance of iron precipitation would significantly aid in the effective implementation of *in situ* bioremediation processes at a larger number of sites.

2.2.2 Nutrient Transport

Existing techniques for formulating and adding inorganic nutrients to the subsurface for *in situ* bioremediation are relatively crude and, ultimately, are based on empirical observations made during and after nutrient additions. However, the chemical interactions between the subsurface soils and the ammonia and orthophosphate that are typically used to satisfy the nutrient requirements of the subsurface microorganisms are very complex. Improved protocols for evaluating soil/nutrient interactions, and identification of alternatives for formulating and adding nutrients could help to prevent precipitation problems and improve process efficiency at many sites.

2.2.3 Hydrogeological Characterization

The development of a detailed, three-dimensional understanding of hydraulic conductivity and geological heterogeneity at a site is critical to the effective implementation of *in situ* bioremediation; however, techniques for evaluating hydrogeological conditions at sites have not evolved in response to the increasingly requirements and applications of these data. The development of rapid, comprehensive, and cost-effective site characterization techniques would significantly improve the ability to evaluate and design *in situ* bioremediation approaches for complex sites.

A more complete understanding of the ways in which soil heterogeneities affect subsurface remediation processes will also enable the design of more effective strategies for *in situ* bioremediation. For example, can sites with contaminants in low permeability layers be more effectively treated through intermittent pumping and injection, to allow time for the diffusion of contaminants out of low permeability zones, and the diffusion of oxygen into these zones? Will the use of high oxygen concentrations increase the driving force for the diffusion of oxygen into the low permeability zones? In addition to improving the design of *in situ* bioremediation approaches, consideration of these types of questions may also lead to the development of more realistic expectations for remediation system performance.

2.2.4 Permeability Enhancement Techniques

Hydraulic fracturing of the saturated zone and pneumatic fracturing of the unsaturated zone have been suggested as techniques for artificially increasing the permeability of fine-grained subsurface soils, thereby enhancing the performance of *in situ* remediation approaches. Pneumatic fracturing for vapor extraction has been field-tested on a limited basis. Both fracturing techniques may potentially extend the effective application of *in situ* bioremediation to sites where poor permeability would otherwise eliminate consideration of the technology; however, considerable additional study is necessary to clearly identify the appropriate applications, limitations, and costs of these permeability enhancement techniques for *in situ* bioremediation.

2.3 PROCESS MONITORING AND DOCUMENTATION

2.3.1 Validation of the Occurrence of *In Situ* Bioremediation

The demonstration and monitoring of *in situ* contaminant biodegradation is critically important for optimizing engineered *in situ* bioremediation systems, as well as for establishing the occurrence of contaminant biodegradation under ambient conditions. There are currently no generally accepted protocols or data that can be used to verify and/or quantify the existence of *in situ* contaminant biodegradation in either saturated or unsaturated subsurface soils. Practitioners of *in situ* bioremediation have commonly used a combination of data to provide an indication of contaminant biodegradation, including microbial analyses, laboratory biodegradation studies, field observations of relationships between contaminant concentrations and oxygen (or other electron acceptors) levels, and temporal trends of contaminant concentrations at appropriate observation points. Nevertheless, there remains a significant need for developing a protocol for evaluating *in situ* contaminant biodegradation that has been reviewed and accepted by an authoritative body of researchers, practitioners, and regulators.

2.3.2 Prediction of Intrinsic Biodegradation Rates

The role of intrinsic biodegradation is important in the evaluation of the significance of natural contaminant attenuation processes. A computer model, BIOPLUME II, has been developed to simulate the biodegradation of dissolved contaminants under oxygen-limited conditions. However, the usefulness of this model is limited by the current understanding of the processes that control oxygen recharge into aquifers, and by the limited number of sites where the relevance of the model has been verified. Additionally, similar predictive models for biodegradation in the unsaturated zone are not currently available.

2.3.3 Characterization of Total Petroleum Hydrocarbons

A wide variety of analytical methods for quantifying concentrations of total petroleum hydrocarbons (TPH) are available, and different state regulatory agencies have different specifications for TPH analyses. Some techniques may be sensitive to naturally-occurring humic materials in soils and may therefore include organics that are not normally susceptible to biodegradation processes. Most conventional TPH analyses do not provide information on the degree of weathering of a petroleum product, its potential for biodegradation, its leachability, or its relative health risk. The development of standard analytical protocols for

TPH to facilitate evaluation of the potential risks posed by a site, would make possible the prioritization of sites for remedial action. Monitoring of contaminant concentrations during the course of a remediation program could also be focused on those TPH constituents of greatest environmental or health risk. With this approach to contaminant monitoring, it may be possible, for example, to demonstrate that remediation is successfully reducing concentrations of leachable hydrocarbons despite the persistence of elevated concentrations of *total* petroleum hydrocarbons. It is important, therefore, that standardized protocols be developed for characterizing petroleum hydrocarbon contamination at sites, establishing appropriate cleanup objectives for these sites, and monitoring the progress of remediation.

2.4 ENGINEERING OPTIONS

2.4.1 Aquifer Aeration

Although aquifer aeration is increasingly being used at hydrocarbon contaminated sites to enhance oxygen transport into the saturated zone, little is known about the efficiency of oxygen transfer into the soil/groundwater matrix, or the physical/chemical parameters that impact this transfer efficiency. Additionally, proven techniques for predicting the effective area of influence created by individual aeration wells are currently lacking. Finally, standardized approaches for empirically determining the area of influence from aquifer aeration systems need to be established in order to optimize the engineering design process of these systems.

2.4.2 Oxygen Diffusion Through Subsurface Soils

The role of diffusion processes in controlling oxygen transfer into lower permeability saturated and unsaturated soils, and in controlling oxygen transfer between the unsaturated zone and the saturated zone, are poorly understood. To what extent does increasing oxygen concentration gradients increase the rate of diffusion-driven oxygen transfer processes? A better understanding of the importance of diffusion processes in regulating the rate and extent of contaminant biodegradation in the subsurface would lead to the development of more effective and economical approaches for introducing oxygen into contaminated subsurface environments.

2.4.3 Use of Surfactants

The use of surfactants is often suggested as a means of "flushing" adsorbed hydrocarbons or occluded hydrocarbon product from soils, thereby transferring the contaminants into the aqueous phase where they can be recovered through conventional hydraulic approaches. However, surfactants typically are composed of organic detergents, and the biodegradation of residual concentrations of these materials remaining in the soil matrix after surfactant flushing may create a substantial oxygen demand, which may reduce the rate at which contaminants of concern are biodegraded. Some surfactants are also reported to cause inhibitory or toxic affects in microbial populations. Carefully controlled, systematic, laboratory and field studies of surfactant flushing, and its integration with *in situ* bioremediation processes, are generally lacking. An improved understanding of the effectiveness and limitations of surfactant use may enhance the rate and extent of free-phase product recovery, especially in fine-grained soils.

2.4.4 Predictive Tools for the Design and Evaluation of Bioventing Systems

Although vapor extraction and bioventing have been practiced for several years, there is an absence of generally accepted vapor and air-flow models for computer simulation of well- or trench-air extraction/injection systems. Additionally, theoretical models, as well as standardized laboratory protocols, to estimate the rate of remediation achievable through bioventing approaches, are lacking. The development of accepted predictive tools for bioventing processes would help establish a basis for these system designs.

2.4.5 Control of Temperature for Enhanced *In Situ* Bioremediation

Temperature is an important variable for biological processes. However, few *in situ* bioremediation projects have been performed in cold weather climates, and little is known about the extent to which contaminant biodegradation rates are impacted by low ambient temperatures. Still fewer projects have attempted to increase temperatures during saturated or unsaturated zone bioremediation to enhance the rate of contaminant biodegradation. Techniques for heating the subsurface, including steam injection, hot water injection, hot air injection, and radio-frequency heating, have been pilot tested, although virtually nothing is known about the affect of these techniques on subsurface biological activity. Additional research is needed to evaluate the costs and potential enhancements to *in situ* bioremediation processes resulting from the application of subsurface heating techniques.

3.0 LITERATURE REVIEW

This literature review provides information to support the technical assessment of the application of *in situ* bioremediation by the railroad industry. The review is consistent with the decision framework that is presented in Figure 4-1 of the User's Guide, in that it groups the information that is relevant to each major decision point in determining the feasibility of using *in situ* bioremediation. This chapter briefly discusses the scope of the literature review, describes the technical approach used to conduct it, and summarizes the results and trends that were identified. Lastly, the implications of these observations on the use of *in situ* bioremediation by the railroad industry are presented.

3.1 SCOPE OF REVIEW

In situ bioremediation, as defined in section 1.1 of the User's Guide, is the focus of this literature review. The primary source of contaminants of concern for this review are petroleum or petroleum-derived products. These contaminants are targeted since they are managed by the railroad industry in significant quantities and their presence in soils and groundwater has historically represented an environmental problem for the industry. The *in situ* bioremediation of other contaminants in groundwater and soils, such as halogenated hydrocarbons, radionuclides, and inorganic compounds, are not within the scope of this literature review.

3.2 TECHNICAL APPROACH

Both published and unpublished literature have been included as part of this literature review (Table 3-1). The review of the published literature was designed to encompass the time period from 1974, when the first successful field application of *in situ* bioremediation was documented in the literature, to the most recent present-day applications. This time period has been addressed by two critical literature reviews. The first of these critical reviews was published in 1988 and was conducted by researchers from the National Center for Groundwater Research at Rice University and the Robert S. Kerr Environmental Research Laboratory of the U.S. Environmental Protection Agency (Lee, et al. 1988). The second of the critical reviews was completed in August 1992, by the University of Illinois and the University of North Dakota with funding from the Gas Research Institute (GRI, 1992). These two documents were then supplemented by other key references which provided specific enhancements to the available information.

TABLE 3-1
Supplementary Literature

Staps, J. J. M. (January 1990). *International Evaluation of In Situ Biorestoration of Contaminated Soil and Groundwater.* National Institute of Public Health and Environmental Protection, Bilthoven, Netherlands, Report No. 738708006.
National Groundwater and Soil Remediation Program (GASReP) (December 1990). *Literature Review and Scoping Study for the Enhancement of the Volatilization of Organic Contaminants in the Unsaturated Zone.* Unpublished Report, No. 90-90-04, Environment Canada, Burlington, Ontario.
Leahy, J. G. and R. R. Colwell. "Microbial Degradation of Hydrocarbons in the Environment." *Microbiological Reviews*, September 1990. Atlas, R. M. "Microbial Degradation of Petroleum Hydrocarbons: An Environmental Perspective," *Microbiological Reviews*, March 1981. These two articles focus on the composition of petroleum hydrocarbons and the microbial biodegradation of the various classes of chemicals that are present.
Staps, J. J. M. (1990). *International Evaluation of In Situ Biorestoration of Contaminated Soil and Groundwater*, Report No. 738708006, National Institute of Public Health and Environment Protection, Bilthoven, The Netherlands. This reference summarizes 23 case histories of the application of *in situ* bioremediation in the Netherlands, West Germany, and the United States. As such, it provides an international perspective to the primary references which focused almost exclusively on efforts in the United States; and
Madsen, E. L. "Determining *In Situ* Biodegradation: Facts and Challenges," *Environmental Science and Technology*, 25(10):1663-1673. This article identifies the difficulties associated with using laboratory data to evaluate full-scale *in situ* bioremediation and discusses the inadequacy of most of the field demonstration tests of this approach to bioremediation.

The source of the unpublished literature was a survey questionnaire that was sent to approximately 38 environmental contacts within the railroad industry. As part of this questionnaire, each individual was asked to describe any applications of *in situ* bioremediation which they had used to address the management of groundwater or soils contaminated with petroleum hydrocarbons. Responses were received from a total of 18 of the individuals, representing a response rate of approximately 47 percent. This information was supplemented with additional information from unpublished sources which has been acquired over the past several years from work conducted with the railroad and petroleum industry.

Lastly, an extensive program has been underway by the U.S. Air Force for the investigation of the *in situ* bioremediation of hydrocarbon contamination (specifically, JP-4 jet fuel) in the unsaturated zone. The results of these efforts will be published in the near future and will no doubt be a valuable companion to this document (U.S. Air Force, 1993, Draft). In addition, the National Academy of Science has published a textbook titled *In Situ Bioremediation: When Does It Work?* (Commission on Engineering and Technical Systems, National Research Council, 1993, National Academy Press, Washington, D.C.). This book focuses on two critical facets of bioremediation: (1) the use of microorganisms to remove contamination from groundwater and soils that remain in-place (i.e., *in situ*) and (2) the methods and procedures to evaluate when an *in situ* bioremediation process is working or has worked. These references will add important information which is relevant to the application of *in situ* bioremediation technology to contamination problems within the railroad industry. Each of these referenced documents have been reviewed and the technology considerations are consistent with this report.

3.3 SUMMARY OF RESULTS AND TRENDS

3.3.1 Chronological Development of *In Situ* Bioremediation

The potential for biodegradation of organic compounds in contaminated aquifers was first recognized in 1971 when it was observed that groundwater contaminated with gasoline from a leaking pipeline contained bacteria that degraded hydrocarbons (Lee et al., 1988). In 1973, a review was published by the Robert S. Kerr Environmental Research Laboratory which served as a basis for the initiation of a fundamental research program on groundwater microbiology (Dunlap and McNabb, 1973). This research focused on the investigation of the existence of subsurface microorganisms and the documentation of their metabolic activity and their nutritional diversity.

One of the first patents for *in situ* bioremediation was issued in 1974 for a process to remove hydrocarbon contaminants from groundwater by stimulating the indigenous microbial population with nutrients and oxygen (Lee et al., 1988). That same year, the first successful application of *in situ* bioremediation documented in the literature was to a gasoline spill from a pipeline leak in Ambler, PA. (Rittman, 1992). In addition to this first application, approximately twenty other field applications of *in situ* bioremediation were cited for the period from 1975 through 1986 in Lee (Lee et al., 1988). Of these, over half were for the management of gasoline or other products refined from petroleum.

There is evidence that the number of field applications of *in situ* bioremediation have continued to increase since 1985, although this cannot be confirmed by sighting any single reference. Nevertheless, case histories provided in Rittman (Rittman, 1992) and Staps (Staps, 1990) indicate the continued field investigation of this approach to soils and groundwater remediation. Furthermore, it is evident in the literature that advances in the technology have also continued. For example, in 1986, a patent was issued for the use of hydrogen peroxide to stimulate the biodegradation of organic contaminants in the subsurface (Lee et al., 1988). The patent described several formulations of nutrients and hydrogen peroxide solutions and processes that can stabilize the decomposition of hydrogen peroxide, control movement of the solution through the aquifer, remove metal ions from the subsurface which catalyze hydrogen peroxide decomposition, and disrupt biofilms that form at the point of injection.

The potential economic advantages of *in situ* bioremediation will likely lead to its continued investigation and development in the future. The ability to avoid excavation and/or removal of the contaminated media, its repeated handling during treatment, and its movement and placement in a final disposal area is a strong incentive to conduct research to overcome any technical limitations associated with the application of the technology.

3.3.2 Biodegradability of Contaminants

Petroleum crude oils are very heterogeneous by nature with the specific chemical composition of the oil depending upon, among other factors, its age and the geologic formation from which it was recovered. Similarly, the refined products of petroleum, such as kerosene, gasoline, gas oils, and lubricating oils, will also be quite variable, with their composition dictated by the crude oil itself as well as the process that was used to refine it. However, generally speaking, all petroleum hydrocarbons will contain some or all of three classes of chemical compounds: aliphatics, aromatics, and asphaltic or polar compounds (Atlas, 1981; Leahy and Colwell, 1990). The aliphatic compounds can be further divided into three groups consisting of alkanes (saturated, straight- or branched-chain hydrocarbons), alkenes (unsaturated straight- or branched-chain hydrocarbons) and alicyclics (saturated and unsaturated hydrocarbons characterized by the presence of closed rings). Examples of these compounds are pentane (normal alkane), 2-Methylpentane (branched alkane), pentene (alkene), and cyclohexane (alicyclic). The aromatics include such chemicals as benzene, toluene, ethylbenzene, and the polynuclear aromatic hydrocarbons. The asphaltic or polar compounds are sometimes subdivided into two groups: asphaltenes (e.g., phenols, fatty acids, or ketones) and resins (e.g., pyridines, carbazoles, or quinolines) (Rittman et al., 1992).

For the most part, aerobic degradation of the aliphatic and aromatic hydrocarbons has been documented. The rates of degradation have been observed to vary with the chemical structure of these hydrocarbons, with the rate decreasing in the following manner: normal alkanes > branched alkanes > low molecular weight aromatics > cycloalkanes. The biochemical fate of the asphaltic fraction of the hydrocarbon is not well understood due to the difficulty associated with the analytical measurement of these compounds (Atlas, 1981). Nevertheless, there is some evidence that the asphaltenes are co-oxidized in the presence of the normal alkanes (Leahy, 1990). Stated differently, while the bacteria utilize the normal alkanes as a primary substrate, oxidation of the asphaltenes has also occurred. Co-oxidation is also believed to play a role in the biodegradation of the complex branched alkanes, as well as some of the alicyclics compounds, since it has been very uncommon to find aerobic bacteria that are capable of growing on these hydrocarbons (Atlas, 1981). Little has been reported in the literature regarding the treatment of these hydrocarbons by anaerobic bacteria, although one reference noted the successful treatment of alkylbenzenes using anaerobic bacteria (Thomas and Ward, 1989). The comparative lack of literature on anaerobic processes relative to aerobic processes for petroleum hydrocarbons implies that this approach to biodegradation is significantly lagging in its development.

The variability of the degradation potential and rates of the components of the crude oil has been evident in the observations of the biodegradation of crude oils: heavy oils (No. 6 fuel oil) have been less responsive to biodegradation than lighter oils (No. 2 fuel oil), and weathered oils have been less responsive than non-weathered or fresh oils (Atlas, 1981). The implications of these observations on the application of *in situ* biodegradation to crude oils or their derivatives is significant from two perspectives. First, it is evident that the degree of treatment that can be achieved for any given petroleum product will depend heavily upon the ratios of the various groups of chemicals that are present. For example, the greater the amount of asphaltics present, the lower the biodegradation potential, and the higher the residual concentrations of the hydrocarbon in the treated product. Secondly, most biodegradation treatment operations will be managed on the basis of the measurement of "total petroleum hydrocarbons" (TPH). There has been a great deal of effort expended to determine the correlation between TPH and the specific chemical constituents of the petroleum (Calabreze et al., 1991). However, since the work-to-date has not provided a definitive correlation, it is not clear how the presence of any one class of chemicals in the hydrocarbon mixture will influence the TPH measurement. In other words, if asphaltic compounds are not measured by the available methods for TPH, their presence and overall resistance to biodegradation would have little or no effect on the assessment of the

biodegradation of the mixture if TPH measurements are used as the basis for the assessment.

3.3.3 Microbial Populations in the Subsurface Environment

Aerobic bacteria and fungi are the primary microbes that degrade petroleum hydrocarbons (Leahy and Colwell, 1981). A majority of these microorganisms is present in the subsurface on the surface of the solids (i.e., attached), with counts that are one to three orders-of-magnitude greater than the bacteria that are free-living in the groundwater (Rittman, 1992). There is little variation in the numbers of attached bacteria within and apart from the sources of contamination, whereas substantially fewer bacteria are present in the groundwater as the distance from the contaminants increases. It is important to note that not only do the numbers of bacteria differ between the solid and water phases, but the bacteria have different morphology (i.e., form and structure) and physiology (i.e., organic processes and phenomena of an organism) (Rittman et al., 1992). However, in general, most of the microbial population in the subsurface is oligotrophic; that is, they are deficient in nutrients (Lee et al., 1988).

The biodegradation of petroleum hydrocarbons in the subsurface requires that the available microbes either have, or acquire, the genes that are necessary to accomplish the treatment. Alternatively, microbes that are known to contain the desired genes can be placed into the subsurface. If the microbes have the proper genetic structure at the outset, then it may be enough to simply stimulate their growth through the addition of nutrients or oxygen, or both, to overcome deficiencies of these materials in the subsurface environment (Rittman et al., 1992). Should this genetic structure be absent, it can be acquired through mutation of the microbes which occurs as a result of the continued use of the contaminant as a food or energy source. However, since the process of mutation is relatively slow and little is known about its application in the subsurface, augmentation of the subsurface microbial population has been attempted by introducing microbes with the appropriate genetic requirements into the subsurface environment. This augmentation can occur in one of two ways. First, if the contaminant is present in sufficient concentrations, the microbes will reproduce and flourish. However, in many cases, there is not a sufficient concentration of contaminant to support this level of growth, and the microbial population does not increase but eventually diminishes and disappears. It should be noted that if the contaminant is only a secondary substrate for the microbes, then it may be possible that microbes with the proper genetic structure can be maintained even at conditions of low contaminant concentration. Under these circumstances, the question is whether a proliferation of the

biomass with the proper genetic coding will occur even though the contaminant is only a secondary substrate. The second means of bioaugmentation involves the transfer of genes from the added bacteria to the existing bacteria through a process known as conjugation. Conjugation involves the transfer and replication of genetic material (i.e., DNA plasmids) through direct contact of the microbial cells. The transportation of the genetic material from one cell to another is under intense investigation but at this time only represents a "promising concept" (Rittman et al., 1992).

The transport of microbes that are added to the subsurface represents a major issue for the use of bioaugmentation in this environment. It is suspected that the microbial addition will not be capable of moving far from the point of injection, since it is likely that the microbes will be filtered from moving liquids by the pore structure of the subsurface geology. Even if the microbes are part of the liquid phase, the movement of the liquids may be very slow and may inhibit their effectiveness. Others suggest that movement of the microbes may not be an issue, since it can occur through enhancements that are made possible by the addition of chemical agents (i.e., chemotaxis) or through rapid cell division and growth on the solid surface (Rittman et al., 1992). One approach to resolving the transport of the bacteria is to directly add the water-soluble enzymes that are secreted by the bacteria during biodegradation instead of adding the bacteria themselves. This option is also subject to the limited velocity that can be achieved by the groundwater, and there may be additional issues associated with the stability of the enzyme in the environment. For example, the enzyme may be altered by the pH or solute concentrations in the groundwater, or the microbes in the subsurface may act on the enzyme and use it as a primary or secondary substrate (Lee et al., 1988).

Most of the discussion has focused on aerobic organisms, i.e., the presence of oxygen is required. In an oxygen-depleted environment, "anaerobic" microbes and processes become operative with the sequence progressing from denitrification to iron and manganese reduction, to sulfate reduction, to methane formation (methanogenic conditions). During this progression, both the redox potential and the energy production decrease. Accordingly, these processes require less nutrients and produce less biomass. By their nature, these processes generally proceed at slower rates than the aerobic processes, may mineralize contaminants that are not treated by aerobic microbes, and will generally require less management. While there has been evidence in the laboratory that phenolics and BTEX have been treated under methanogenic (anaerobic) conditions, the general statement in the literature is that *in situ* bioremediation using anaerobic microbes, alone or in combination with aerobic microbes, is still in the developmental stages (Lee et al., 1988).

The literature generally concludes that the stimulation of indigenous aerobic microbes probably offers the greatest potential to generate a subsurface microbial population that can contribute to the *in situ* bioremediation of hydrocarbons. Natural mutation processes are too slow and the benefits of bioaugmentation, through microbial or enzyme addition, are not clear at this time. Due to the issues associated with the survival of the microbes and enzymes and their transport in the subsurface, the data suggest that this approach will have limited value for *in situ* bioremediation.

3.3.4 Low Concentration Effects

The natural systems in the subsurface environment, by their very nature, tend to have low concentrations of the reactants and catalysts that are required for bioremediation. Specifically, the concentrations of the contaminants or microbial substrate, the electron acceptor, or the essential nutrients are often deficient and have the potential to inhibit *in situ* bioremediation.

Contaminant Concentration

There are steady-state contaminant concentrations below which the subsurface microorganisms will not grow. If the microbial mass does not grow, it does not consume the contaminant or substrate and no further reduction in contaminant concentrations can be achieved. This may or may not be a problem, depending upon the contaminant concentrations which must be achieved by the remediation action. For example, if the minimum achievable contaminant concentration is greater than the desired clean-up goal, it may not be possible for *in situ* bioremediation to remediate the site.

This minimum achievable concentration can be overcome if non-steady state conditions exist; that is, if conditions exist such that there is a net decay of the biomass. While undergoing a net decay, or reduction in thickness, the biomass still utilizes the contaminant and thereby reduces its concentration to less than the previously defined minimum. Since biofilms persist for some time after they are established, a biofilm will continue to remove the contaminant, no matter how low the concentration, until the biofilm disappears from the system (Rittman et al., 1992).

This low concentration limit may also be overcome if the contaminant is a secondary substrate for the microbes or if cometabolism occurs. In the first situation, the biomass

growth occurs through the utilization of a primary substrate that is present in abundant supply. At the same time, the microbes continue to utilize the contaminant as a secondary substrate to support some portion of its growth. The continued growth of the biomass results in the continued reduction of contaminant concentrations to levels that are below the steady state minimum that would exist were it the primary substrate for the microbes. If cometabolism is occurring, the contaminant is transformed, incidentally, by microbes that normally transform a different, but structurally similar, compound. Since the growth of the microbes do not depend in any way on the utilization of the contaminant, that is, the microbes grow by utilizing only the structurally similar compound, the concentrations of the contaminant can be reduced to below any threshold concentration (Rittman et al., 1992).

Low-limit threshold concentrations of the contaminant can still exist even under non-steady state conditions in natural systems. These conditions are related to the presence of subcritical amounts of biomass and lack of enzyme induction. If the initial amount of microbes present is too small, the kinetics of biodegradation will not be rapid enough to produce measurable removal of the contaminant. Similarly, the microbes may not produce sufficient enzymes to degrade the contaminant unless it is present in concentrations that are sufficient to make its metabolism worthwhile.

Electron Acceptor Concentration

Oxygen is the electron acceptor in the aerobic biodegradation process. If it is not present in sufficient concentrations, which is often the situation in the subsurface environment, it will limit the growth of the microbes and, hence, the degradation of the contaminant substrate. Any deficiency in oxygen concentrations in the subsurface is usually addressed by the injection of air, i.e., air sparging, or the addition of hydrogen peroxide. Air sparging, depending upon the temperature of the groundwater, can provide as much as 8 to 12 mg/l of oxygen to the aqueous phase. However, hydrogen peroxide is infinitely soluble in water and its limits for providing oxygen are driven by its toxic effects on the microbes. For example, a 3 percent hydrogen peroxide solution is a sterilant and as low as 200 mg/l in solution can be toxic to microorganisms. Furthermore, at concentrations of greater than 100 mg/l, the hydrogen peroxide may degas, form air bubbles, and plug the pores of the subsurface. For the most part, the correct dosage of hydrogen peroxide must be experimentally determined for each site-specific situation (Lee et al., 1988).

The movement of the hydrogen peroxide solutions through the subsurface environment must be controlled to provide oxygen concentrations throughout the contaminated zone. Methods

which have been used to accomplish this focus on the alteration of the viscosity, interfacial tension, and density of the solution. Viscosity changes are induced through the addition of hydratable polymers such as polysaccharides or polyacrylamides. These materials increase the viscosity of the solution, which slows its movement and reduces the rate of diffusion. The addition of surfactants decreases the interfacial tension and disperse the materials throughout the contaminated region. Lastly, salts such as sodium chloride and calcium chloride are used to increase the density of the solution (Lee et al., 1988).

Other methods for adding oxygen to the subsurface include the sparging of pure oxygen, the introduction of ozone, soil venting in the unsaturated zone, and the use of colloidal gas aphrons (i.e., a colloidal dispersion of air in a surfactant solution). With the exception of soil venting, none of these techniques have been used with any frequency. Soil venting typically involves the withdrawal of vapor from the unsaturated zone using vapor recovery wells. Alternatively, the reverse process, or air flooding, involves the increase in soil gas flow by injecting air into the vadose zone through air inlet wells. In both cases, the increase in soil gas flow makes more oxygen available to reaerate the groundwater and to fill the pore space of the unsaturated soil (Lee et al., 1988).

Nutrient Concentrations

The concentrations of carbon, nitrogen, phosphorous, hydrogen, sulfur, and selected trace metals must be sufficient to permit the biological reactions to occur (Lee et al., 1988). The concentrations that are required is specific to each situation and would have to be determined experimentally using laboratory flask studies. These laboratory studies were routinely conducted in the early days of the development of *in situ* bioremediation with the goal of optimizing the nutrient blend. Over time, as more and more data were acquired, the optimization of these nutrient additions was assigned a lower priority since it was not evident that their impacts on the biodegradation process were significant. However, the presence or lack of effects of the nutrients on *in situ* bioremediation has not been methodically studied nor quantitatively defined as of this time.

3.3.5 Accessibility of Substrate

The contaminant may be present in the subsurface but may be not biodegradable because it is not accessible to the microorganisms. This lack of accessibility can usually be attributed to two factors: (1) sorption of the contaminants to the solid surfaces and (2) low solubility of the contaminant in water. Other, less important factors, which can also result in the lack

of accessibility of the contaminants are restricted transport into the cells, complexation with naturally recalcitrant molecules, and inaccessibility of cleavage sites for the extracellular enzymes. Sorption and solubility are discussed in more detail in the remainder of this section.

Sorption

There is little information regarding the effects of a solid surface on microbial activity. Studies have shown mixed results indicating positive, negative, and no effects depending upon the microorganism, the surface, the contaminant, and the phenomenon of interest. It is known, however, that the solid surfaces can affect bioremediation by influencing the availability of the contaminant to the microorganisms. Specifically, it is understood that sorption to the solid surfaces can decrease the concentration of the contaminant in the aqueous phase and retard its biodegradation. In this situation, the biodegradation rate becomes a function of the mass transfer of the contaminant from the solid to the aqueous phase which, in turn, is a function of the surface area of the solid. In some cases, this sorption can be irreversible and totally prohibit the biological treatment of the contaminant. On the other hand, sorption can actually enhance the effect of biological treatment for the treatment of toxic contaminants. This occurs because the sorption onto the solid prevents the contaminant from reaching concentrations in the groundwater that are toxic to the organisms. As such, the rate of release of the contaminant into solution is sufficiently slow to permit the biodegradation reactions to occur in the aqueous phase before the toxic concentrations are achieved (Rittman et al., 1992).

It should be noted that there are other solid phase interactions besides sorption that can play a role in the chemical or biological reactions of the contaminants in the subsurface. These include cation exchange capacity of the soils, the organic content of the soil, the internal pore structure of the solids, and the chemistry of the clay constituents. However, these factors are typically less important because they are associated with solid phases found in geologic zones that have hydraulic conductivities (K) that cannot support *in situ* processes, i.e., $K > 10^{-5}$ m/s. In other words, many of these factors are associated with clays and silty sands that are not good candidates for *in situ* bioremediation because of their hydraulic conductivity properties (Rittman, et al., 1992).

Aqueous Solubility

Petroleum hydrocarbons can exist as pure phase hydrocarbons in the subsurface environment. These non-aqueous phase liquids (NAPLs) can exist as residual saturation in the soil, as pools on the top of the water table (light non-aqueous phase liquids or LNAPLs), or at the bottom of the aquifer (dense non-aqueous phase liquids or DNAPLs). In all of these cases, the solubility of a specific chemical component is a function of its mole fraction in the mixture and its pure component solubility in water. Furthermore, the rate of biodegradation of the NAPL is a function of the ability of the microbe to take the hydrocarbon phase into the cell.

The possible mechanisms for the uptake of the hydrocarbon include: (1) direct contact between the cells and large (> 1 mm diameter) hydrocarbon droplets (macroemulsions), (2) direct contact of the cell with hydrocarbon droplets of submicron size (microemulsions), otherwise known as pseudosolubilization, and (3) uptake of hydrocarbons that are dissolved in the aqueous phase. Emulsions can be formed by agitation of the petroleum/water mixture, the use of synthetic surfactants or microbes that secrete extracellular biosurfactants, or modification of the cell surface during growth. Since, in the natural environment, there is usually a low microbial density and limited emulsification, it is probably necessary for the microbe to adhere to the NAPL to achieve growth. For this reason, it would seem that the direct contact mechanisms would be the dominant mass transfer mechanisms for the hydrocarbon to enter the cell. It should also be noted that in an open system, the microbes must also adhere to the substrate, since it is likely that the substrate will be continually moving and could diffuse away from the microbial population (Rittman et al., 1992).

Since mass transfer limitations may limit the biodegradation of hydrocarbons in some instances, means of increasing the interfacial surface area will have a tendency to increase the rate of biodegradation. Increase in the surface area can be accomplished by altering the microbial population of the petroleum itself. The petroleum can be altered through the use of surfactants, dispersants, or emulsifiers; however, most field applications of these techniques have actually resulted in a negative effect on the rate of biodegradation. In fact, in some laboratory studies, the addition of the surfactant actually resulted in the clogging of the pores of a soil column. The addition of these additives to the subsurface also has the effect of mobilizing the contaminants and permitting or enhancing their migration. Microbial reactions can have a similar effect by producing metabolites such as alcohols, phenols, and organic acids, all of which are more mobile that the parent petroleum hydrocarbon. As such, any time that these additives are used, precautions must be taken

to control the movement of the contaminants so they do not spread beyond the current impacted site (Lee et al., 1988).

3.3.6 Contaminant/Metabolite Toxicity

The subsurface environment can be toxic to the microorganisms depending upon the chemical characteristics of the contaminant and the physiology of the microorganism. This can result in the inhibition of microbial growth and consequently substrate consumption.

The most common mechanisms that cause toxicity involve interference with the metabolic processes by inhibiting enzyme activity or non-specific interactions with the microbial cells. Examples of the latter are the presence of the copper or mercury cation, both of which bind to the cell and cause it to leak fluids. Although the toxicity mechanism for petroleum hydrocarbons is not well understood, it does appear that it is related to non-specific cell interactions. Specifically, it has been documented that normal alkanes with less than nine carbon atoms in length, as well as the C5 through C12 aliphatic alcohols and acids which are metabolites of the biological oxidation of the short chain normal alkanes, have all demonstrated some toxicity to microbial populations. It is believed that this effect is a result of the influence of these chemicals on the permeability barrier of the cell, and the disruption of the hydrophobic interactions that occur within and through the cell wall (Rittman et al., 1992).

The toxicity of the aliphatic acids, alcohols, and alkanes is directly related to their solubility, with the toxicity increasing with solubility; that is the acids are more toxic than the alcohols, which are more toxic that the alkanes. For any given homologous series, such as the aliphatic acids, both solubility and toxicity increase as the chain length decreases. Similarly, for aromatic hydrocarbons, the toxicity involves interactions with the cell membranes and increases with an increase in solubility. It is interesting to note that there are some inherent conflicting properties of chemicals beyond solubility which influence their toxicity. For example, for most chemicals, their "inherent" toxicity increases as their molecular weight increases and their solubility decreases. However, at the same time, their maximum achievable concentration in aqueous solution decreases as their solubility decreases. Consequently, it is not unusual for the most toxic compound is a homologous series to be a compound with intermediate solubility (Rittman et al., 1992).

In summary, while toxicity mechanisms are known to exist and have been documented, toxicity has not been a major issue during the application of *in situ* bioremediation. The

primary exceptions have been when the subsurface environment has gone septic or when the initial concentrations of the contaminant have been so excessive that it has overwhelmed the bacteria. In these instances, more global site-wide actions have to be taken prior to the initiation of *in situ* bioremediation, i.e., the bulk of the free phase hydrocarbon must be removed from the subsurface.

3.3.7 Design Considerations

There are several design features which must be considered to properly apply *in situ* bioremediation at a site. The first of these is the determination of the basic design parameters such as the biodegradation kinetic rate and the oxygen and nutrient requirements. These parameters are generally determined through treatability studies. On the basis of the treatability studies, a conceptual design can be prepared that is based primarily on the biological degradation characteristics of the solid-contaminant matrix (EPA 1991; GRI, 1992). To properly complete the design and to implement the system in the field, the treatability information must be combined with site characterization data that describe the hydrogeology at the site, as well as the nature and extent of contamination. These hydrogeological factors determine if the proper reactants can be provided to the proper place in the subsurface at the proper time. Lastly, there are numerous engineering factors related to the selection of the proper equipment and its installation in the field.

Treatability Studies

Treatability studies are required to develop the technical basis for a design of any *in situ* bioremediation effort. Typically, these studies follow a progression of levels, each one increasing in complexity while providing more and more information related to the final design of the system.

Generally speaking, the first level treatability study focuses on the ability to biodegrade the contaminant. These studies can be conducted in serum bottles or flasks and involve the incubation of the solid-contaminant matrix. During incubation, contaminant concentrations are monitored with time, as are the consumption of oxygen and other parameters that provide an indication that biological degradation is occurring. In essence, this study reveals if the potential for bioremediation exists for the solid-contaminant matrix of interest.

The next level of treatability study investigates the conditions at which biodegradation will occur and initiates optimization of the process. These studies emphasize the determination

of reaction rates, mass transfer rates and the influence of oxygen, nutrients, pH and other factors on those rates. Many times, the physical configuration of the proposed treatment system is simulated in an attempt to reflect the influence of the system geometry on the chemical and biological processes that are in action. For example, it is not uncommon for soil column studies to be used when the intent is to investigate *in situ* treatment of the contaminants. Alternatively, pan reactors are often used to simulate prepared-bed or land treatment, and laboratory liquid/solid slurry reactors are used to emulate their large scale counterpart. However, recognizing the difficulties associated with extrapolating laboratory results to predict the treatment that will be achieved in the field, both the U.S. Environmental Protection Agency and the Gas Research Institute have developed laboratory protocols to screen the use of biological treatment for a solid-contaminant matrix (EPA, 1991; GRI, 1992). Both methods are designed to provide rapid methods for evaluating the applicability of biological treatment. The EPA protocol documents that the contaminant is susceptible to biological treatment whereas the GRI protocol emphasizes an assessment of the treatment endpoint that is achievable using biological treatment, regardless of how or where it is applied. In essence, the EPA protocol focuses on the microbiology while the GRI protocol focuses on an examination of the combined effects of biological, chemical, and physical processes and their impact on the achievable treatment endpoint.

The last level of treatability study involves an assessment of the site characterization data and the execution of a field pilot study to examine the ability to implement *in situ* bioremediation on a full-scale basis. Historically, these field tests have been inadequate in proving that *in situ* biodegradation of organic contaminants has occurred. This is a direct result of the inability to control a field site and its inherent heterogeneity, as well as the inability to conduct mass balances and to quantitatively identify the microbiological contributions to contaminant loss. As such, guidance has been provided regarding the execution of a step-wise strategy for determining *in situ* bioremediation processes (Madsen, 1991). The steps and their primary objectives and approaches are listed in the following:

- Conduct preliminary site investigation - This effort should assess the spatial, physical, and hydrologic constraints for successfully understanding the site. The type and degree of contamination that has occurred and the predominant physiological regimes that are already established by the indigenous organisms should be identified;

- Develop *in situ* biodegradation assessment strategy - Using the existing knowledge of biochemistry, the key chemical and biological criteria that match site characteristics with the biodegradation processes should be identified. For example, the presence of toxic chemicals in high concentrations may preclude biodegradation entirely or restrict it to zones where these contaminants have been diluted;

- Conduct detailed site characterization - The site characterization sampling plan should be designed to provide optimally interpretable chemical and microbiological measurements. As such, trends indicative of on-site processes can be discerned from the data;

- Seek proof of *in situ* biodegradation - This is a critical step which seeks to: (1) distinguish biotic from abiotic processes by seeking chemical and biological criteria that are uniquely indicative of microbial metabolic processes, (2) identify the production of metabolic intermediate compounds as they contrast with the contaminants originally released at the site, (3) examine the selective disappearance of microbiologically labile compounds especially as they contrast with conserved nonbiodegradable tracers that are fortuitously present at the site, (4) determine the loss of coreactants such as oxygen or nitrate, (5) identify metabolic adaptation based upon a comparative analysis of assays from samples within and outside of the zones of contamination, and (6) manipulate the field site to elicit unique biotic responses such as the addition of nutrients or other key metabolic stimulants to a portion of the site; and

- Gather microbiological evidence that confirms *in situ* biodegradation - Use convergent lines of indirect evidence to lend strength to arguments that suggest the occurrence of *in situ* biodegradation. Examples of indirect evidence include laboratory tests that demonstrate that biodegradation occurs in biotic but not abiotic samples from the site, the isolation and enumeration of microorganisms from the site that catalyze biodegradation, and use of DNA or RNA gene probes to assess distribution of potential catabolic expression.

Even with the application of these strategies to the implementation of field studies of *in situ* bioremediation, the documentation of *in situ* biodegradation is difficult and will rarely be quantitative. Hence the problems of extrapolating laboratory data to the field will continue to be a problem into the future.

Hydrogeological Factors

The ability to deliver reactants to and retrieve degradation products from the subsurface environment is critical to the successful application of *in situ* bioremediation. The hydrogeological factors of importance include knowledge of the consolidated and unconsolidated geologic zones, the presence of fractures in the bedrock, the thickness and extent of the aquifer, the location of geologic confining units, the depth to the water table, and the particle size distribution and solid/liquid interfacial area. However, the most critical hydrogeological factors are the hydraulic conductivity, porosity, and hydraulic gradient. These factors dictate the quantity and velocity of fluid flow in the subsurface (Rittman et al., 1992).

Clogging of the pores in the subsurface can interfere with the ability to conduct any *in situ* remediation strategy. Clogging can result from a combination of biochemical and geochemical (or precipitation) reactions that result from the injection of liquids with or without additives into the subsurface aquifer. For example, it is possible that the microbial mass or the byproduct offgases of the biological reactions can obstruct the interstices of the porous media. Furthermore, the rheological properties of the pore fluids may be altered by bacterial metabolites. In addition, mineral precipitates can form when the inorganic solids and groundwater of the subsurface are contacted with the injected fluids. The end result of these processes are inhibited flow patterns which cannot efficiently support *in situ* bioremediation (Rittman et al., 1992).

Engineering Factors

Since the movement of fluids through the subsurface is so central to the application of *in situ* bioremediation, the most critical engineering design factors are associated with the injection, subsurface control, and recovery of the aqueous and hydrocarbon liquids. The first consideration is the injection of fluids into the subsurface. Both injection wells and infiltration galleries are usually considered for this application. However, the latter is used nearly twice as much as the former. This is partially attributable to the fact that infiltration galleries are required for conducting *in situ* treatment in the unsaturated zone (Staps, 1990).

Design of injection and recovery systems requires the specification of the number and location of the injection points, the establishment of an injection sequence including the time variability of the injection rate, and the determination of extraction rates. These factors can be established only with the application of numerical flow models, contaminant

transport models, and optimization models. Generally, all of these models are available but are in varying stages of development. The need for better and more accurate models of the subsurface exists, to ensure success for *in situ* bioremediation (Rittman et al., 1992).

Another key engineering factor is well maintenance. Following installation, it is important that the injection and recovery systems remain free and clear for optimal movement of fluids. This task is complicated by the fact that the *in situ* bioremediation process is designed to stimulate microbial activity and establishes situations where precipitation reactions are likely. Under these conditions, it is necessary to consider acid treatments, mechanical brushing, and high flow rate injections as means to avoid and/or address the plugging problems (Rittman et al., 1992).

Lastly, the design of any above-ground treatment systems is also an important consideration. These systems can range from simple oil/water separation units that multiple treatment units to address volatile organic compounds, PAHs, and cyanide. These systems are generally compact and self-contained and tend to be small, portable, and modular.

3.3.8 Overview of Relevant Field Applications

The field applications of *in situ* bioremediation which were identified in the literature have been summarized in Chapter 5, the Literature Review, of the User's Guide. Over 50 applications were identified, of which approximately 12 were overseas in Germany or the Netherlands. Of the 40 that took place in the United States, eight were conducted within the railroad industry, as indicated by the survey that was conducted of the members of the American Railroad Association. Field applications of soil vapor extraction, as applied to petroleum products, were also included in this review, since this approach is often used as a component of *in situ* bioremediation of the vadose zone. Its purpose is to oxygenate the vadose zone as well as to assist remediation by enhancing the removal of the more volatile contaminants such as benzene or toluene.

The information that is provided in the Literature Review of the User's Guide includes the following areas:

- Site Description;
- Site Location;
- Contamination Type and Volume;
- Soil and Geology;

- Oxygen Source;
- Other Additives (e.g., Nutrients or Inoculum);
- Duration of Remediation;
- Design Concept; and
- Results.

A review of this information reveals that there are several common features associated with the field applications of *in situ* bioremediation. These common features are highlighted in this section with an emphasis on the types of facilities, the types of contaminants, the target remediation goals, and the basic design features of the system. The intent of this review is to provide a hands-on perspective of the research issues based upon the state of the art of the technology as it is practiced in the field.

Facility Type

In general, nearly all of the field applications in the United States and abroad have occurred at fueling stations, sites of leaks and spills of hydrocarbons, and at general industrial areas where hydrocarbons or solvents have been managed. The applications in the railroad industry reflect this general trend although there is a predominance of applications at fueling facilities (5 of 8 applications that were identified).

Nature and Extent of Contamination

The types of contaminants which have been targeted by *in situ* bioremediation projects are primarily petroleum-based hydrocarbons and chlorinated or other volatile organic compounds. Gasoline is by far the most common petroleum-derived material that is cited in the literature, followed by diesel oil, fuel oil, and aviation fuels. Also noted, but much less frequently, are mineral oil, creosote, and non-chlorinated solvents. Applications in the railroad industry almost exclusively address diesel oil and fuel oil.

A wide range of initial contamination concentrations were encompassed in these applications as shown in the following:

Initial Contaminant Concentrations in Soil

Total Petroleum Hydrocarbons: 2000 mg/kg
Gasoline: 2000 to 7000 mg/kg

Fuel Oil: 3000 to 20,000 mg/kg
Diesel Oil: 10,000 mg/kg
Aromatic Hydrocarbons: 60 to 700 mg/kg
Aliphatic Hydrocarbons: 600 to 2000 mg/kg
BTEX: 110 to 5600 mg/kg
Mineral Oil: 200 to 600 mg/kg

Initial Contaminant Concentrations in Groundwater

Dissolved Hydrocarbons: 20 to 30 mg/l
Gasoline: 30 to 40 mg/l
Aliphatic Hydrocarbons: 2 to 3 mg/l
Aromatic Hydrocarbons: 5 to 170 mg/l
Chemical Oxygen Demand: 170 to 20,000 mg/l

The specific analytical techniques that were used to determine the soil and groundwater concentrations were not specified in the literature. As such, it is not possible to compare these contaminant concentrations with one another, since it is not known how much they overlap in their coverage of specific hydrocarbon compounds. Nevertheless, the information indicates that *in situ* treatment has been applied to hydrocarbon concentrations in the soil as high as 2 percent (20,000 mg/kg), and in the groundwater, to 0.02 percent (170 mg/l).

Remediation Goals and Treatment Endpoints

It is uncommon for remediation goals to be specified for *in situ* bioremediation applications. In all of the field applications that were cited in the literature, there were only three cases where there were any specific goals or targets for the remediation effort. One of the projects identified the recovery of a specific quantity of free product as the remediation target, whereas the other two specified groundwater quality limits. In one case, the groundwater quality limit was 0.72 μg/l of benzene while in the other case the Federal Drinking Water Quality Standards were identified as the remediation goal. (It should be noted that one field application that took place in the State of New Jersey did not meet the remediation goals that were established for the project; however, the literature did not identify what the limitations were). No remediation goals were specified for any of the presented applications of *in situ* bioremediation in the railroad industry.

In spite of the fact that few remediation goals were established, a variety of treatment endpoints for the soil and groundwater were reported for the various field applications. While it is important to understand the various conditions under which these endpoints were achieved, the range of observations that were recorded in the literature are summarized below:

Final Contaminant Concentrations in Soil

Total Petroleum Hydrocarbons: No Data
Gasoline: <10 to 2900 mg/kg
Fuel Oil: < 20 to 1200 mg/Kg
Diesel Oil: 4600 mg/kg
Aromatic Hydrocarbons: <0.005 mg/kg
Aliphatic Hydrocarbons: No Data

Final Contaminant Concentrations in Groundwater

Dissolved Hydrocarbons: 0.6 to 2.0 mg/l
Gasoline: < 1.0 mg/l
Aliphatic Hydrocarbons: 0.1 to 0.7 mg/l
Aromatic Hydrocarbons: 0.08 to 1.5 mg/l
Chemical Oxygen Demand: 20 to 30 mg/l

More details regarding the actual treatment conditions that resulted in these treatment endpoints are provided in the User's Guide. However, it may be necessary to examine the original reference to obtain a complete appreciation of what was done and how the data were generated and analyzed.

It should be noted that the treatment endpoint that is achievable using biological treatment of any type is a strong function of the specific characteristics of the soil-contaminant matrix. Accordingly, the ability or lack of ability to achieve a specific contaminant concentration in one case does not necessarily imply that it will or will not be achieved in a totally different situation.

Basic Design Features

The most prevalent design for *in situ* bioremediation involves the recovery of free product, followed by groundwater recirculation, which includes surface treatment and the addition of additives to the recovered groundwater prior to its injection into the subsurface. Free product recovery is achieved using the strategic placement of recovery wells. Groundwater is recirculated using the same recovery wells and infiltration surface trenches for its injection into the subsurface. Injection trenches are preferred over injection wells because they permit the treatment of contamination in the vadose zone. Groundwater treatment generally includes oil and water separation followed by either air stripping, carbon adsorption, or biological treatment. The latter is often used when it has been determined that microbial addition into the subsurface would be desirable. In Germany and the Netherlands, the offgas from air strippers is treated using soil biofilters. This technology is currently under investigation in the United States and has not been used extensively in commercial applications. Additives that are often added to the groundwater prior to injection include nutrients to supply nitrogen and phosphorus, and microbial inoculum, if not provided by the surface biological treatment system.

The next most common application is the direct injection of air and other additives, such as nutrients or surfactants, into the saturated zone of the subsurface. No movement of the groundwater is required as part of this approach and the air and additives, which are identical to those identified above, are usually introduced into the subsurface using injection wells. It is not uncommon to link this approach, commonly called air sparging, with bioventing of the vadose zone.

3.4 DATA GAPS AND RESEARCH NEEDS

There are four major areas where fundamental research is required to further advance the potential for *in situ* bioremediation. These areas are: (1) quantification of biodegradation kinetics, (2) dissolution and desorption kinetics of poorly soluble substrates, (3) biological induced clogging of the subsurface, and (4) the transport of colloids. Other, more general issues of concern, include the development of methodologies and techniques to characterize the effectiveness of treatment, the examination of risk-based treatment endpoints, and the definition of the limits of the applicability of the technology.

3.4.1 Quantification of Biodegradation Kinetics

There is a direct need to quantify the kinetics of biodegradation in order to permit the effective design of an *in situ* bioremediation process. To accomplish this, the efforts must focus on the separation of the abiotic and biotic processes and the derivation of kinetic rate constants for aerobic, denitrifying, sulfate-reducing, and methanogenic conditions. Research should also be directed towards the development of an understanding of the effects of inhibitors, electron donors, and electron acceptors on the biodegradation kinetics. Lastly, consideration should be given to the ecological impacts of the degradation of trace components in the environment and the shift in the genetic population of indigenous microbial populations.

3.4.2 Dissolution/Desorption Kinetics

Mass transfer may often be the limiting factor for *in situ* bioremediation and the kinetics of contaminant removal will be dictated by dissolution and desorption kinetics. While there has been some research on this topic, there remain several unknowns. It is necessary to develop the baseline kinetics for these processes for petroleum hydrocarbons, alone and in mixtures. Methodologies for this have been developed at the University of Florida and the University of Texas and these methodologies can be applied to petroleum hydrocarbons. In addition, the ability to enhance the baseline kinetics through the use of additives such as surfactants must be better understood including the secondary effects of these additives, e.g. toxicity to the microbes.

3.4.3 Subsurface Clogging

The mechanisms of clogging in the subsurface are not fully understood and represent a potentially significant barrier to conducting *in situ* bioremediation. As such, research is required to determine the primary biological and geochemical mechanisms for clogging, determining the rate of clogging that can occur, and examining methods and techniques for its prevention, minimization, and/or management.

3.4.4 Transport of Colloids

The microbial populations and some substrates are in the form of colloids in the subsurface. The transport of these materials in the subsurface is not well understood and should be investigated further. Of particular interest with regards to bacterial transport are the roles

of surface adhesion, chemotaxis, and growth along the solid surfaces (biofilm chemotaxis). Adhesion is also a potential problem for substrates, as is the potential for the hydrolysis. The latter becomes even more of an issue when the colloidal substrate acts as a facilitator for the transport of adsorbed metals or organic contaminants.

3.4.5 Other Potential Research Topics

Characterization of Treatment Effectiveness

Operating in the subsurface environment makes it difficult to characterize the effectiveness of the treatment, which is required to demonstrate to the regulatory agencies and the public that the contaminants have been destroyed and no longer present a threat to the public health or the environment. Since it is impossible to sample a site to fully address this problem, it is imperative that the proper indirect evidence be gathered to support the concept that adequate treatment has occurred. This evidence includes a mixture of laboratory studies, rigorous chemical and biological site characterization studies, and a multidisciplinary examination and interpretation of the data. At the same time, the use and development of quantitative modeling tools to assist this effort could prove helpful in the interpretation and analysis of the data.

Risk-Based Endpoints

It is understood that bioremediation processes cannot achieve as low a treatment endpoint as some other processes such as thermal treatment. Furthermore, it is understood that *in situ* bioremediation may not be as effective as *ex situ* treatment approaches due to mass transfer limitations and other factors that are introduced in the subsurface environment. Nevertheless, it can be argued that the treatment that is received is adequate because it addresses the public health and ecological risks that were present at the site prior to treatment. This risk-based approach to site management is imperative if the use of *in situ* bioremediation is expected to continue and to flourish. However, the methodology to assess the public health and ecological risks associated with contaminated media is not well developed and is currently the subject of other research programs. The results of this research must be integrated into the technical investigations of *in situ* bioremediation to ensure that the proper issues are being addressed during the implementation of this remediation strategy.

Definition of Limits of Applicability

Research is required to develop a protocol to determine the applicability of bioremediation, in general, and *in situ* bioremediation, specifically, to the contaminated media at a specific site. The protocol should be inexpensive and rapid and should permit the rejection or acceptance of *in situ* biological treatment as early in the feasibility study process as possible. It should also provide for the investigation of enhancements for those instances where conventional approaches to treatment may not be sufficient. GRI has initiated the development of such a protocol, and its application to petroleum hydrocarbons of concern to the railroad industry should be evaluated.

4.0 REGULATORY ISSUES REQUIRING FURTHER RESEARCH

In situ bioremediation is a remediation technology that has considerable promise and is being studied closely by government, industry and academia in order to increase its effectiveness and applicability. "Bioremediation is a technology that is gaining momentum in technical, policy and popular circles. It is also a technology associated with mystery, controversy and 'snake oil salesmanship'" (*In Situ Bioremediation, When Does It Work?* National Research Council, National Academy Press, 1993). *In situ* bioremediation has potentially wide applicability to railroad soil and groundwater remediation. However, there are several difficult regulatory issues that often prevent its selection and limit its implementation. Many regulatory agencies and officials are suspect of the technology's ability to perform at all, and others question its ability to achieve the remediation goals established for soils and groundwater. This section briefly summarizes a series of topics which are potential areas for future research and which may offer opportunities to improve regulatory understanding and acceptance of the capability and control of *in situ* bioremediation. The issues have been grouped into six general categories:

- Environmentally Acceptable End Points;
- System Performance Measurement;
- Site and Contaminant Characterization;
- Delivery of Additives to Subsurface;
- Case Studies;
- Expert Systems;

This effort, and thus the associated research efforts, are specifically focused on diesel fuel contamination.

4.1 ENVIRONMENTALLY ACCEPTABLE END POINTS

Defining the end points for bioremediation in general, and for *in situ* bioremediation in particular, is one of the most controversial and difficult issues for railroad environmental officials and environmental regulatory personnel to deal with. The question of "how clean is clean" is slowly evolving into the question of "how clean is clean enough." Both state and federal agencies are attempting to answer this question, most notably the EPA.

The states all have developed cleanup standards for both soils and groundwater. Some states are in the process of defining site-specific, risk-based standards for soil and

groundwater remediation efforts after having attempted to use technology-based, universal standards. Recent regulatory efforts at the federal level have attempted to address the issue of clean-up levels of contaminated soils. The Federal EPA is moving in an entirely different direction by attempting to redefine hazardous waste and establish universally applicable treatment standards for hazardous soils. All of which indicates that the issue of defining the performance goals for remediation efforts in general, and *in situ* bioremediation efforts specifically, is a technically and regulatorily complicated area. Industrial research efforts can be effective in developing information that can be used by regulatory agencies in establishing reasonable standards, as well as information that can be used by railroad environmental professionals in responding to regulatory agency proposed site specific standards.

There are four general areas of interest that relate to the definition of environmentally acceptable standards: mobility of the contaminants in the environment, toxicity of the contaminants to human and ecological receptors, loss of contaminant mass due to bioremediation and how that loss impacts both the mobility and toxicity, and the site-specific contaminant exposure mechanisms and routes.

4.1.1 Mobility

The mobility of diesel fuels in the soil and groundwater are of concern from a regulatory point of view, since migration of petroleum constituents is the primary source of risk associated with these materials. Mobility of petroleum constituents is heavily dependant on the soil's matrix within which they are contained, and the physical properties of the contaminant. Heavier fractions of petroleum products are normally less mobile than the lighter fractions. Weathering and biological activity associated with bioremediation have an impact on the mobility of petroleum contaminants by initially reducing the lighter more mobile fractions. In addition, there is some evidence to indicate that biostabilization of the heavier fractions contributes to a reduction in their mobility. Research that focused on understanding the mobility of petroleum constituents in soils, and the effect of biological activity on that mobility, would be useful in understanding the capabilities of bioremediation, designing full scale systems, and developing information necessary to establish realistic performance standards for bioremediation systems.

4.1.2 Toxicity

The toxicity of petroleum constituents in soils varies with contaminant age and the degree of biological degradation that has occurred. Very little data exist that document the toxicity

of weathered and biologically-treated petroleum contaminated soils. Data that document the reduction of toxicity as compared to the reduction in chemical concentrations of petroleum constituents would be useful in defining performance end points for bioremediation systems.

4.1.3 Loss

The biokinetics of diesel fuel degradation in not completely understood. Specifically, the relationships between rate constants and treatment end points, soil types, age of contaminants, and oxygen and amendment concentrations are not understood. Much of the engineering of full scale systems is based on rules of thumb and past experience. In order for environmental regulatory agencies to be comfortable with *in situ* bioremediation, a much more rigorous and well documented basis must exist for the design and implementation of the technology. It is desirable to develop empirical and theoretically based engineering standards for the design of *in situ* systems. Bench-scale treatability studies designed to develop these data would be useful in addressing their need.

4.1.4 Exposure

There is a growing trend on the part of regulatory agencies (especially at the state level) to use site-specific relative risk assessment procedures as a method for arriving at clean-up goals for remediation efforts. Much is unknown about how bioremediation, and *in situ* bioremediation in particular, impacts the toxicity and mobility of petroleum (diesel) constituents, and the associated risk of untreated soils or residual levels after treatment. An effort should be focused to discover how degradation of specific compounds occurs and the relationship between initial concentrations and final concentrations. Chromatographs of diesel contamination before, during, and after treatment would yield a better understanding of the impact that bioremediation has on the contaminant toxicity.

4.2 SYSTEMS PERFORMANCE MEASUREMENT ISSUES

A predominate research issue is related to how best to measure and document the degradation of contaminants of concern (diesel) by *in situ* remediation systems. Because of the difficulty of gathering truly representative samples of the contaminated media, some surrogate for contaminant levels is needed to monitor system performance and document the treatment end point. As discussed in the technology-driven research of Chapter 2, and the literature-driven research of Chapter 3, some generally accepted protocol is needed that

will verify the occurrence and effectiveness of *in situ* bioremediation. There is also a need for research that helps to define parameters that can easily be measured and yet be truly indicative of the systems' performance in reducing the contaminant levels.

TPH Measurement Issues

Total Petroleum Hydrocarbon (TPH) is commonly used as a regulated parameter for monitoring biosystems and determining when the site is deemed "clean." It is widely known that TPH is not a completely appropriate parameter to be applied to diesel fuel contamination because the analytical standards apply to gasoline and other ranges of hydrocarbons. The EPA standard analytical protocols for TPH require the use of a "standard" based on gasoline, distillate fuels and residual oils. This "mixed" standard results in inaccurate measurement of diesel fuel contamination. Therefore, there is a need to develop a database of information that can be used by railroad environmental officials when discussing and negotiating clean-up standards for diesel contaminated soils. The type of information would include comparisons of the levels of particular petroleum constituents to TPH levels. These comparisons could then be useful in understanding the toxicity of treated and untreated soils relative to specific petroleum constituents and how those levels relate to, if at all, the TPH concentrations in the same soils. Pre- and post-biotreatment analysis of soils using TPH and constituent-specific protocols would be useful. A literature review and some diesel-specific laboratory studies that address the reproducibility of TPH data, and how diesel-specific TPH data relate to typical state TPH clean-up standards would be useful. Much of this work is being done by The American Petroleum Institute and may not require a parallel effort on AAR's part.

4.3 CHARACTERIZATION

One of the primary issues identified by regulatory agency personnel in helping them evaluate the appropriateness of *in situ* bioremediation is the a complete and accurate site description or characterization. Such a site description needs to include information about soils, groundwater, contaminant characterization and site setting. Environmental regulators see themselves as needing information sufficient to "agree that the technology proposed has a reasonable chance to reduce the contaminant(s) at the site to acceptable levels" (National Research Council, 1993). There are several guidance documents at both the state and federal levels which define the scope of a Phase 1 or a Phase 2 Site Assessment. This guidance is normally based on the need to define the extent of the problem as opposed to gathering information needed to define and engineer a solution.

In order to better define the extent and magnitude of contamination of a site, and to effectively define and engineer an appropriate remediation solution, development of a standard protocol for site characterization is needed. In addition, investigative techniques, sampling protocols and statistical approaches to handing site characterization data need to be developed.

4.4 DELIVERY

The effective delivery of oxygen and treatment amendments to the subsurface is an issue of both technical and regulatory concern. Regulators need to be assured that these essential materials are delivered to the area of contamination and that they are effectively used by an appropriate microbiological community to degrade the contaminants of concern. Research is needed to define alternate techniques for the delivery of these materials in different soil types. This research is similar to the research defined in the technology section of the User's Guide, as well as the means identified in Chapter 2 and Chapter 3 of this Research Guide.

4.5 CASE STUDIES

There is a continuing need on both the railroads' and regulatory agencies' parts to have current and accurate information about the use of *in situ* bioremediation and its effectiveness. The respondents to the survey that was conducted as part of the effort to prepare the User's Guide, indicated that obtaining information about successful implementation of *in situ* bioremediation was a high priority. Limited information was gathered concerning 26 uses of bioremediation and six uses of *in situ* bioremediation by the railroad industry in the U.S. Because of the limited scope of this survey, the information generated concerning these railroad applications was very confined. No technical or regulatory details on the applications were solicited or documented.

A more intense effort to gather detailed information about actual railroad and other related industrial projects (such as petroleum refining and marketing soil remediation efforts) that have successfully utilized *in situ* bioremediation would be useful to the railroad industry. The information could be used to define the design basis for successful systems, as a screen for identifying qualified contractors and products, and as a database for developing information to convince state and Federal regulators that site-specific applications of *in situ* bioremediation are effective.

The U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Technology Innovation Office (TIO) has an ongoing program to gather and disseminate information about bioremediation. Specifically, TIO publishes, on a regular basis, a list of active and completed bioremediation projects that includes, for each site, the type of contaminants, contaminated media, and technology configuration. TIO has also published, and updates, the Bioremediation Resource Guide (EPA/542-B-93-004) which includes a listing of resource documents, databases, and dockets that "have the potential to ease the implementation of bioremediation at hazardous waste sites" (EPA, 1993). EPA also includes bioremediation in the SITE's program, which is a program designed to implement, at full scale, innovative remediation technologies and make performance data from these technologies available to the public (EPA, 1993). This database, combined with more detailed information gathered for the railroads and related industries, could be used as the basis for a detailed case-study effort. Finally, the EPA Office of Research and Development and the Office of Solid Waste and Engineering Response publish a series of bulletins titled "Bioremediation Field Initiative" which document the technical and regulatory details of the use of bioremediation at Superfund and RCRA sites. This effort provides contacts and sources for additional, detailed information. The EPA-published Bioremediation Resource Guide is also a source of technical information related to bioremediation.

4.6 TECHNOLOGICAL DECISION MAKING (EXPERT SYSTEMS)

An expert system as it applies to environmental remediation technology is an information network that is available to practitioners to obtain information about past performance of the technology and the engineering of a new system. Such a system would identify individuals and organizations who have experience in the use of bioremediation. A railroad-specific expert system would insure that railroad-specific application would be documented and accessible. This expert system is similar to the compilation of case studies discussed in Section 4.5 except that an expert system would provide more detail about the technology used and would allow access to individuals and firms with expertise in those technologies.

As railroad environmental professionals seek to implement *in situ* bioremediation, they often seek information about contractors, products and methods that have proven to be successful. The case studies discussed above could be used as a source of such information, but a more detailed and formal expert system would be useful in providing access to information and expertise in the bioremediation field. EPA has developed several databases (discussed in

section 4.5 above) that are accessible to the public and these could be used as a model to help define an expert system for *in situ* bioremediation.

5.0 SUMMARY

Each section of the Research Guide (Technology, Literature, and Regulatory) has identified *in situ* bioremediation research needs that are applicable to the railroad industry. This chapter presents a summary of these topics in a format that can be used by the AAR in evaluating and prioritizing the railroad industry's research needs and efforts. Several of the research needs are already being addressed by other trade groups or EPA, and an additional, independent effort by AAR would most likely not be productive. The railroad industry's most effective approach in these cases would most likely be to monitor, review and advise or co-fund/participate in these efforts.

In some cases, other parties are conducting research which is not focused on the railroad industry's specific interests and problems, although the *general* research area has been identified. In those cases, AAR's efforts might be to cooperate with other researchers and develop an independent yet closely coordinated effort.

Finally, some cases are so particular to the railroad industry or are of such importance to the industry, that a truly independent effort would be justified.

The research needs identified by this report are each summarized by stating the unknowns (What do we not understand?), the goals (What information would come out of research?), the benefits (How would the information derived from the research benefit railroad practitioners?), and options for execution (What options are available for conducting research?).

IN SITU BIOREMEDIATION RESEARCH SUMMARY

Subject: Subsurface Clogging

1. **What do we not understand?**
   Both biological and geochemical reactions can lead to the formation of precipitates. These precipitates can obstruct the flow of liquids in the subsurface and eventually lead to the complete stoppage of flow. More information is required to define the biological and geochemical mechanisms that are responsible for the formation of precipitates, to determine the rates of these reactions, and to examine methods and techniques for their prevention, minimization, and/or control.

2. **What information would come out of research?**
   The research would provide the information that is necessary to minimize the formation of precipitates in the subsurface during the implementation of *in situ* bioremediation.

3. **How would the information derived from the research benefit railroad practitioners?**
   The ability to minimize precipitate formation in the subsurface will increase the effectiveness and lifetime of an *in situ* bioremediation effort.

4. **What options are available for conducting research?**
   This research can be conducted in the laboratory, although eventually field data will be required for its confirmation and/or verification. Similar research is underway in the area of oil and gas exploration and production where the formation of radioactive scale in the surface and subsurface equipment is of a great concern. It may be possible to translate the research protocols of this industry to the subsurface problems that are faced by the railroad industry; however, it is likely that independent research will be required to apply these protocols to sites of the railroad industry.

IN SITU BIOREMEDIATION RESEARCH SUMMARY

Subject: Quantification of Biodegradation and Desorption/Dissolution Kinetics

1. What do we not understand?

Both abiotic and biotic reactions are occurring simultaneously during the bioremediation of groundwaters and soils. For example, the mass transfer of the contaminants from the soil to the water either through desorption or dissolution is occurring simultaneously with biodegradation. The presence of these multiple, concurrent reactions makes it difficult to isolate the reactions and to quantify their kinetics. Hence, the relative rates of desorption, dissolution, and biodegradation are not known for the major contaminants.

2. What information would come out of research?

Reaction kinetics for the desorption, dissolution, and biodegradation of individual contaminants will be quantified under a variety of conditions.

3. How would the information derived from the research benefit railroad practitioners?

In situ bioremediation systems can be better designed and optimized with an understanding of the kinetics of the governing reactions which include contaminant desorption, dissolution, and biodegradation.

4. What options are available for conducting research?

Several organizations such as the EPA and GRI have ongoing efforts to quantify these reaction kinetics. The research involves laboratory experiments and it is likely that AAR could piggy-back onto these programs and incorporate soil-contaminant matrices that are specific to the railroad industry into the research protocols that have been developed by these other groups.

IN SITU BIOREMEDIATION RESEARCH SUMMARY

Subject: Surfactant Additions

1. **What do we not understand?**
   The ability of surfactants to reduce the soil contaminant concentrations in the subsurface beyond that which is achievable using conventional *in situ* bioremediation has not been demonstrated.

2. **What information would come out of research?**
   Biodegradation rates and treatment end points will be quantified with and without the addition of surfactants and a comparison will be made to determine the impacts, if any, of surfactant addition on the capabilities of *in situ* bioremediation.

3. **How would the information derived from the research benefit railroad practitioners?**
   The use of surfactants may provide the capability to achieve lower soil contaminant concentrations using *in situ* bioremediation. This enhancement would be achieved by increasing the desorption of the contaminants from the soil matrix into the aqueous phase.

4. **What options are available for conducting research?**
   Laboratory research studies are required to determine the effects of surfactants on the treatment end points that are achievable using *in situ* bioremediation. EPA and others have research protocols available in this area which may be capable of providing the desired information for the railroad industry.

IN SITU BIOREMEDIATION RESEARCH SUMMARY

Subject: **Subsurface Transport**

1. **What do we not understand?**
   The transport of the contaminants and reactants, e.g., oxygen, microbial mass, and nutrients, in the subsurface is not well understood. Specifically, it is not clear how far from the point of injection that the reactants will move before they are made unavailable through both biotic and abiotic reactions.

2. **What information would come out of research?**
   The abiotic and biotic reactions of the key reactants in the subsurface will be identified and the kinetics of these reactions will be determined.

3. **How would the information derived from the research benefit railroad practitioners?**
   The reaction and kinetic data can be used in subsurface flow and transport models to aid in the prediction of the movement of the reactants in the subsurface. The ability to predict the movement of these key reactants in the subsurface will permit the design of improved *in situ* bioremediation systems and the optimization of the usage of the key reactants.

4. **What options are available for conducting research?**
   This research is best conducted in the laboratory, although the extrapolation of the results to field conditions is an important and difficult step. This can best be done by conducting field demonstration tests, using models to predict the results based upon the laboratory-derived coefficients, and comparing predicted and actual results and assessing their implications on the accuracy of the laboratory-derived coefficients.

IN SITU BIOREMEDIATION RESEARCH SUMMARY

Subject: Biomass Production and Nutrient Requirements

1. What do we not understand?

The need for nutrient addition to stimulate bioremediation is generally understood but the relationship between the levels of nutrient addition and the occurrence of biological reactions and their rates is not known. Furthermore, the extent and rate of biomass production during the microbial degradation of contaminants has not been documented.

2. What information would come out of research?

Relationships between the types and levels of nutrient addition and the occurrence and rates of contaminant biodegradation will be defined, as will the rate of biomass production during microbial assimilation of contaminants.

3. How would the information derived from the research benefit railroad practitioners?

The research results will permit the optimization of nutrient additions into the subsurface to yield the maximum rates of biodegradation while minimizing the expense associated with these additives. Optimization will involve the development of a biomass that is sufficient to biodegrade the contaminants but that does not result in the plugging of the subsurface pore space.

4. What options are available for conducting research?

Laboratory studies are required to develop the desired relationships. Every attempt should be made to conduct these studies with the geologic materials and groundwater from the specific site.

IN SITU BIOREMEDIATION RESEARCH SUMMARY

Subject: Alternate Electron Acceptors

1. **What do we not understand?**
   The occurrence and rate of microbial degradation of contaminants with electron acceptors other than molecular oxygen have not been adequately characterized. Of particular interest are biological reactions that occur in the presence of nitrate-, sulfate-, and carbon dioxide-reducing bacteria.

2. **What information would come out of research?**
   Biological reaction rates and reaction byproducts will be determined using nitrate, sulfate, and carbon dioxide as the primary electron-acceptors.

3. **How would the information derived from the research benefit railroad practitioners?**
   In situ bioremediation in the saturated zone may depend on the ability of bacteria to utilize electron acceptors other than molecular oxygen. Furthermore, the ability to use chemicals such as nitrate as the primary electron acceptor may improve *in situ* bioremediation by enhancing the ability to supply it to the subsurface and to eliminate chemical side reactions that produce insoluble precipitates.

4. **What options are available for conducting research?**
   Laboratory studies are required to develop the desired biological reaction rates and to identify critical reaction byproducts.

IN SITU BIOREMEDIATION RESEARCH SUMMARY

Subject: Bioavailability of Contaminants

1. What do we not understand?

It is believed that contaminants must be present in aqueous solution for microbial degradation to occur. This implies that contaminants with limited solubility in water may not be readily available to participate in biological reactions and, hence, their reduction in biological treatment systems has a maximum limit. The bioavailability of contaminants as a function of the chemical and physical properties of the soil-contaminant matrix has not been investigated for railroad sites. Furthermore, the effect of biofilms, which attach directly to the soil, on the bioavailability of contaminants is not understood.

2. What information would come out of research?

The bioavailability of contaminants in soil-contaminant matrices that are common to railroad sites will be determined as a function of the chemical and physical properties of the soils and contaminants. In addition, the ability of attached biofilms to reduce contaminant concentrations to that below which would be predicted based on bioavailability considerations will be determined.

3. How would the information derived from the research benefit railroad practitioners?

The bioavailability of contaminants may define the minimum concentrations that can be achieved during the *in situ* bioremediation of contaminated soils. An understanding of these limits will permit the acceptance or elimination of this technology for a specific site application based on the desired remediation goals. It may also be true that the lack of bioavailable contaminants in a soil-contaminant matrix that has been biologically treated, will support the acceptance of alternative concentration limits for soils on the basis that these treated materials represent *deminimus* risk to the environment and human health.

4. What options are available for conducting research?

EPA and GRI have established laboratory protocols to evaluate the bioavailability of manufactured gas plant (MGP) site contaminants. These protocols can be applied to contaminated soil samples from railroad industry sites. In addition, the laboratory application of a "bioassay" test protocol should be implemented concurrent with the bioavailability laboratory studies to correlate environmental impacts with bioavailability test results.

IN SITU BIOREMEDIATION RESEARCH SUMMARY

Subject: Addition of Alternate Substrates

1. **What do we not understand?**
   The ability of alternate substrates to enhance the biodegradation of target contaminants and/or to permit the achievement of reduced concentrations of contaminants is not understood.

2. **What information would come out of research?**
   Alternate substrates will be identified and correlations will be developed between their concentrations and the biodegradation rates and treatment end points of the target contaminants.

3. **How would the information derived from the research benefit railroad practitioners?**
   Low concentrations of contaminants may not be capable of supporting the microbial population necessary to permit biodegradation to occur. Alternate substrates may provide a mechanism to generate a biological population that is capable of degrading both the substrate as well as the contaminant. The presence of this active biomass has the potential to enhance the biodegradation of the contaminant and/or to reduce the contaminant concentrations to levels below that which is attained without the addition of the substrate.

4. **What options are available for conducting research?**
   Laboratory research studies are required to identify alternate substrates and to determine their impacts on contaminant degradation and end points.

IN SITU BIOREMEDIATION RESEARCH SUMMARY

Subject: Addition of Microbial Products

1. What do we not understand?

The ability to enhance *in situ* bioremediation through the addition of microbial products (e.g., pre-grown microbial mass or bacterial enzymes) has not been demonstrated.

2. What information would come out of research?

The occurrence and rate of biodegradation of contaminants will be measured with and without the addition of microbial products to permit an analysis of the impact of their addition on the capability of *in situ* bioremediation.

3. How would the information derived from the research benefit railroad practitioners?

Substantial claims are made by vendors that microbial additions can be used to stimulate and/or enhance biodegradation of contaminants in the subsurface. The research results will either confirm or refute these claims and will provide industry representatives with the knowledge required to effectively respond to these vendors and to design an optimal subsurface bioremediation project.

4. What options are available for conducting research?

Laboratory research studies and field tests are required to determine the effects of microbial additions on the rates of contaminant biodegradation.

IN SITU BIOREMEDIATION RESEARCH SUMMARY

Subject: Aquifer Aeration

1. What do we not understand?

The efficiency of oxygen transfer from aquifer aeration processes, and the physical/chemical parameters of the subsurface that impact oxygen transfer to the groundwater, have not been explicitly studied.

2. What information would come out of research?

Research into the mechanical and chemical mechanisms involved in aquifer aeration will provide information that can be used to determine optimum air flow rates, spacing of aeration wells, construction techniques for aeration wells, etc. Additionally, data derived from this research will help to identify conditions that warrant consideration of sparging with pure oxygen.

3. How would the information derived from the research benefit railroad practitioners?

Information from this research would facilitate the design of more efficient groundwater aeration systems as oxygen sources for *in situ* bioremediation. Advanced development of such systems may decrease the dependence on groundwater extraction for containment of contaminated groundwater.

4. What options are available for conducting research?

Research on aquifer aeration should be conducted on three levels: laboratory studies to directly measure oxygen transfer in the soil/water matrix; computer modeling studies to identify the influence of key process parameters in the simulation of field-scale systems; and field pilot studies to verify the information derived from computer simulations.

IN SITU BIOREMEDIATION RESEARCH SUMMARY

Subject: Permeability Enhancement Techniques

1. What do we not understand?

The feasibility, limitations, and cost-effectiveness of hydraulic and pneumatic fracturing techniques for increasing the permeability of unconsolidated formulations has not been clearly demonstrated.

2. What information would come out of research?

Demonstration projects that measure intrinsic permeability or hydraulic conductivity in different soil types, before and after pneumatic or hydraulic fracturing, are needed to reveal the benefits and limitations of these techniques.

3. How would the information derived from the research benefit railroad practitioners?

The development of cost-effective permeability enhancement techniques will enable the application of *in situ* bioremediation processes to a wider variety of sites, especially sites that have low natural permeability.

4. What options are available for conducting research?

Permeability enhancement processes require field demonstration using pilot-scale systems at low permeability sites. It will be important to thoroughly characterize the geological and hydrogeological characteristics of the demonstration site, before and after the application of permeability enhancement procedures.

IN SITU BIOREMEDIATION RESEARCH SUMMARY

Subject: Hydrogeological Characterization

1. What do we not understand?

Techniques to characterize geological and hydrogeological heterogeneity are poorly developed, and the impact of such variabilities on bioremediation processes have not been quantitatively assessed.

2. What information would come out of research?

Research in these areas will identify new characterization techniques, applications, and costs, and will provide data demonstrating the relationship between geological/hydrogeological heterogeneity and the performance of bioremediation processes.

3. How would the information derived from the research benefit railroad practitioners?

This research will result in the development of more thorough and economical approaches for characterizing subsurface conditions at sites, and will allow the development of more effective design of *in situ* bioremediation systems to account for site-specific geological/hydrogeological variables.

4. What options are available for conducting research?

Characterization techniques must be developed and proven in the field. Evaluation of the impact of subsurface heterogeneity on remediation performance may be initially performed using predictive computer models, followed by field investigation.

IN SITU BIOREMEDIATION RESEARCH SUMMARY

Subject: Nutrient Transport

1. What do we not understand?

The interactions of nutrient additives with specific soil types, and the transport of these compounds within the subsurface soil matrix, has not bee adequately studied.

2. What information would come out of research?

Measurement of the rate at which different nutrient formulations are transported through columns of different soil types will address nutrient/soil interactions.

3. How would the information derived from the research benefit railroad practitioners?

Research focused on nutrient/soil interactions would facilitate the implementation of more effective *in situ* bioremediation processes with less guesswork by the practitioner. Such research may also produce standardized testing protocols for evaluating nutrient/soil interactions, and selecting appropriate nutrient formulations.

4. What options are available for conducting research?

Laboratory studies are necessary to evaluate the relationship between soil types, nutrient formulations, and rates of nutrient transport.

IN SITU BIOREMEDIATION RESEARCH SUMMARY

Subject: Control of Temperature of Enhanced *In Situ* Bioremediation

1. What do we not understand?

Although subsurface temperatures are relatively consistent and somewhat favorable for biological treatment, theoretically, improved performance could be achieved if elevated *in situ* temperatures could be achieved. We do not understand how to uniformly increase *in situ* temperatures, if such an approach would be effective, and the cost/benefit of such an approach.

2. What information would come out of research?

The effect of the alternatives for *in situ* temperature manipulation on biological activity and treatment effectiveness would be documented. Some evaluations of alternative techniques (steam, hot water, air gas, microwave, direct electrical energy, etc.) would give an indication of what technique would yield the most cost-effective solution.

3. How would the information derived from the research benefit railroad practitioners?

The results could be directly applied by railroad practitioners to *in situ* system design. For example, *in situ* bioremediation could be applied at sites in colder climates where it is not currently considered practical, thus, expanding the utility of the technology.

4. What options are available for conducting research?

Identify other parties who are interested in, or currently involved in, related research and participate in the efforts. Both lab/bench and field-scale treatability studies could be used to help define the effectiveness and cost of temperature control options.

IN SITU BIOREMEDIATION RESEARCH SUMMARY

Subject: Characterization of Total Petroleum Hydrocarbons

1. What do we not understand?

Total petroleum hydrocarbons (TPH) is the most common regulated parameter at hydrocarbon contaminated sites, including diesel. TPH is a broad-banned parameter that uses as standards other petroleum constituents besides diesel. TPH is also sensitive to naturally occurring organics. We do not understand how soils and groundwater (treated and untreated) contaminated solely with diesel respond to TPH analysis.

2. What information would come out of research?

A profile of diesel-contaminated media TPH results would be compared with constituent-specific analysis, yielding a good picture of how TPH analysis compares to total hydrocarbon constituents.

3. How would the information derived from the research benefit railroad practitioners?

When negotiating with regulators, railroad practitioners would be able to site specific data about the validity of TPH as an appropriate parameter for diesel-contaminated environmental media.

4. What options are available for conducting research?

API and others have done significant work in this area, but with an emphasis on gasoline.

IN SITU BIOREMEDIATION RESEARCH SUMMARY

Subject: Risk-based End Points

1. What do we not understand?

The relative toxicity and related risks associated with petroleum-contaminated soils are not well understood. Several states are moving toward risk-based approaches to establishing end points for remediation efforts. Therefore, it is important to understand the mobility and toxicity of treated petroleum-contaminated soils.

2. What information would come out of research?

The relative mobility, toxicity, and the characteristics of bioremediated petroleum-contaminated soils would be determined from literature and/or lab studies and would provide information related to animal, human, and ecological risks associated with these materials.

3. How would the information derived from the research benefit railroad practitioners?

Railroad officials could use the data in negotiations with state and federal officials related to specific site remediation projects. The data derived could be used in various risk-based formula established by states in their programs to establish risk-based site-specific cleanup standards.

4. What options are available for conducting research?

Rely on published data and lab studies performed by others related to toxicity and risk associated with petroleum products in soils.

IN SITU BIOREMEDIATION RESEARCH SUMMARY

Subject: **Case Studies/Expert Systems**

1. **What do we not understand?**
   Although there is a significant amount of bioremediation and *in situ* bioremediation work being conducted by railroad environmental programs, there is not much information available to practitioners about those experiences. There is much data available in the literature about performance of *in situ* bioremediation systems.

2. **What information would come out of research?**
   A frequently updated database on the design and performance of *in situ* bioremediation systems would be provided. The expert system would allow users to have access to railroad personnel who have experience with the use of *in situ* bioremediation, as well as the actual data on system performance. A railroad-specific database would be established and maintained and would be available to railroad practitioners for implementing *in situ* bioremediation.

3. **How would the information derived from the research benefit railroad practitioners?**
   They would be able to use the data in (1) evaluating options for their sites, (2) convincing regulators that *in situ* bioremediation is an effective tool and (3) providing guidance to engineering contractors in the selection and design of full-scale systems.

4. **What options are available for conducting research?**
   Rely on EPA's distribution of treatability data and other information related to bioremediation. Rely on the wide variety of contractors, consultants, and literature sources.

IN SITU BIOREMEDIATION RESEARCH SUMMARY

Subject: Definition of the Limits of Applicability

1. What do we not understand?

Although we understand that *in situ* bioremediation will not be effective in treating all contaminants and will not work due to certain site specific conditions (i.e., low soil permeability) there is not a reliable guide that is accepted by the profession and the regulatory community that defines those limits of applicability.

2. What information would come out of research?

A research effort designed to document the limits of applicability would result in a guide that would define those contaminant and site conditions that would limit or prevent the application of *in situ* bioremediation.

3. How would the information derived from the research benefit railroad practitioners?

The information or guide could be used by railroad practitioners in screening sites for the application of *in situ* bioremediation, as an authoritative source to be used when negotiating with regulators about the application of the technology, and as guidance to contractors who are tasked with the design of systems.

4. What options are available for conducting research?

The User's Guide is a very good prototype of the type of document that is needed. By working with other industrial groups and EPA, a widely accepted document or model could be developed.

IN SITU BIOREMEDIATION RESEARCH SUMMARY

Subject: **Prediction of Intrinsic Biodegradation Rates**

1. What do we not understand?

The role of natural biodegradation is important in evaluating the significance of natural contaminant attenuation processes. We do not understand how hydrocarbons naturally degrade at depth.

2. What information would come out of research?

An understanding would be developed of how natural degradation occurs and how those naturally occurring processes can be used and relied upon to clean up contamination.

3. How would the information derived from the research benefit railroad practitioners?

When defining the need for and scope of remediation efforts, it is useful to understand the effect that natural degradation will have on hydrocarbon contamination. The ability to predict the future behavior of contaminate plumes using more than the hydraulic models currently available would be useful in negotiation with regulatory agencies.

4. What options are available for conducting research?

Look toward other industrial groups who have similar interests and participate in a joint effort to address this issue.

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